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THE JOURNAL OF HYGIENE

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AN INVESTIGATION OF THE PATHOLOGY OF
"GROUSE DISEASE."

A REPORT OF THE INVESTIGATIONS UNDERTAKEN FOR
THE GROUSE DISEASE INQUIRY.

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Plates I—V. Nine Tables.

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Introduction.

THE fact that the lower animals are subject, like man, to diseases does not obtrude itself upon our notice, probably because they often hide themselves when ill, and creep into some corner to die, and perhaps because they have less aptitude for expressing their sufferings. However this may be, they bear so generally the aspect of perfect health that when attacked by a serious epizootic disorder, the latter gets dubbed with the name of the species it attacks; and so one hears of silk-worm disease, horse sickness, or swine fever, as though they were the only diseases from which these species suffer.

The grouse, like other animals, suffers, doubtless, from a variety of diseases and disorders, but one of these, it is held, so far exceeds all the rest in importance that it has earned for itself the name of grouse disease. Sportsmen and gamekeepers bear undivided testimony to the existence of this epizootic, which appears with varying severity and regularity in Spring and Autumn; but it is not easy to be certain that in "grouse disease" we have to do with a specific infectious disease, as is generally assumed, or merely with a concatenation of disastrous consequences set in train by unusual privation, due perhaps to bad weather. Still less easy is it, in the case of any given bird, to tell whether it is suffering, or has suffered from, "grouse disease" or no, especially at a time when birds are dying in unusual numbers; for if we agree that there is a specific infection, we must admit also that privation claims its toll, and which of the two our bird is suffering from is not easy to decide, even after it is dead, for grouse disease has no characteristic symptoms, or very obvious macroscopic lesions.

Our work has been done both with diseased and healthy birds. The former were caught by the keepers in a feeble or dying condition, at times when dead birds were being picked up in considerable numbers on the moor, and it was consequently believed that "grouse disease" was prevalent. But the mortality then was, we believe, not very great, in fact insignificant when compared with the really bad years such, for example, as 1873, and it is, we suppose, just possible that we never came across the genuine epizootic grouse disease at all.

The diseased birds which were subjected to bacteriological examination were nearly all caught alive and brought or sent to the temporary laboratory. Some, of course, died on the journey, but in only a few preliminary instances were cultures made from the latter,

and then only when it was exactly known when the bird was last seen alive.

In addition to these birds from which cultures were made many others, which were picked up dead, were examined for lesions and gross parasites. As will be easily understood the difficulty of obtaining diseased birds alive was very great, and the number investigated therefore very small.

The control observations on normal birds were more numerous; for, through the kindness of Sir Richard Graham, Lord Lovat and others, we had no difficulty in obtaining as many as we wanted. Some few of these were examined in Scotland, but the great majority were received alive in our laboratory at Cambridge. A considerable number of these were caught for us by the keepers, and since it is no doubt easier to catch a feeble bird than a strong one, it may be that they do not fairly represent the average normal bird. It is probable, however, that they do not fall far short, if at all, of this standard, for they were plump and of good weight. They, of course, contained numerous entozoa, as also do the strongest birds which fall to the gun. The rest were hand-reared birds kept in captivity.

The first to attribute grouse disease to a living parasite was, we believe, Cobbold¹ (1873) who drew attention to the presence of the small nematode worm, *Trichostrongylus pergracilis*, often in large numbers in the caeca of grouse which were supposed to have died of the disease. Nineteen years later Klein² (1892) investigated the disease, and came to the conclusion that it was "an acute infectious pneumonia" caused by a specific bacillus, which he found in the blood and organs of birds which had succumbed to the disease. But neither of these theories of grouse disease has found general acceptance. Against Cobbold's view is often urged the well established fact that *Trichostrongylus pergracilis* is present practically without exception in the normal wild grouse, and often too in extraordinary numbers, and it has never hitherto been clearly shown to be more numerous in individuals believed to be suffering from grouse disease than in others. Klein's bacillus on the other hand has long been suspected of being no other than *B. coli*, which, as is well known, rapidly invades the tissues after death. To these points we must return when we have recorded our own observations.

¹ *The Grouse Disease*, The Field Office, London, 1873.

² *The etiology and pathology of Grouse Disease, Fowl Enteritis and some other diseases affecting birds*. 1892. London, Macmillan & Co.

It is necessary at this stage to explain how we came to be associated with the work and what facilities we had for carrying it out.

Grouse disease having been reported in Scotland early in May 1908 we were invited to undertake bacteriological investigations, and accordingly one of us (L. C.) proceeded north to commence preliminary work. Through the kindness of Lord Lovat some rooms at Beaufort Castle were converted into a temporary laboratory, and every effort made by his staff of keepers to procure diseased birds in a living condition. At the same time owners of grouse moors in the neighbourhood were asked to procure living sickly birds if possible. A few days later a move was made to Mr Perrins' moor at Ardross, where Mr Cuthbert, his agent, kindly placed a room in his own house at our disposal for use as a laboratory. The visit terminated after a week as it was necessary to return to Cambridge, but during the time 11 diseased birds and one normal bird were examined together with others picked up dead on the moors. The latter were, of course, useless for bacteriological examination since in the organs of birds which have been dead for some time, whether diseased or healthy, and of birds which have been shot and wounded in the abdomen, *B. coli* and other intestinal bacteria swarm in all the cultures.

A second visit to Scotland was made from August the 25th to September the 1st. During that time eight fresh grouse were examined for bacteria, one being a bird caught when obviously ill on Cawdor moor and received alive.

After this work was continued in Cambridge on normal birds which reached us alive, and on sickly birds caught alive, immediately killed and packed in ice and sent to us from time to time from various moors as occasion offered.

During the visits to Scotland it was, of course, not possible to carry out all the precautionary measures which are described later as having been taken when working in our own laboratories at Cambridge, but the most important of these precautions were observed, such, for example, as plucking the birds before they were brought into the laboratory, the free use of the flame for singeing, and of the actual cautery for destroying any stray particles of feather and for burning the skin through which the incisions were made. In the preliminary experiments also the method of getting at the lungs from behind, which is described later, was adopted; and emulsions of the organs were made between sterilised plates, but the glass frame, in which this was done at Cambridge, had not been adopted at the time of the earlier experiments.

One of the first objects of the experiments was to seek for Klein's bacillus and to compare it with other members of the colon group in the light of the great advances in bacteriology since Klein's observations were made 18 years ago. Our next was to look for characteristic lesions.

The preliminary observations in Scotland showed at once the presence of bacilli of the colon type, which could not be distinguished from Klein's bacillus, in the livers and sometimes in the other organs of diseased grouse, but it soon became evident that these micro-organisms might be present also in the organs of grouse presumably quite normal. No pneumonia was seen in any of the birds examined by us in a perfectly fresh condition, the lungs being always pale pink in colour and free from congestion. In birds picked up dead on the moor it was not always easy to make a definite statement about the lungs as they were often deeply stained and otherwise altered, but in the fresher specimens it was apparent that there was no pneumonia. In the fresh diseased birds the livers were not obviously altered, though in those birds which were picked up dead they often showed more or less of that blackish colour, which has sometimes been described as characteristic of grouse disease, but which is certainly due to post-mortem changes. We had the opportunity of seeing many of these birds through the kindness of Dr E. A. Wilson, who was working at Beaufort at the same time, and it was he who first pointed out to us the entozoal and other parasites of the grouse. He too it was who first pointed out to us that the most notable lesions were in the caeca. The mucous membrane often appeared deeply reddened along the convexities of the longitudinal ridges, and sometimes thickened. To the naked eye, or with the aid of a hand lens, it was plain that considerable pathological change had taken place here, but there was no obvious ulceration. There were always large numbers of strongyli in these caeca. This condition was most advanced in birds which were picked up dead, but it was no post-mortem change, for it was found also in weakly birds which were brought to us alive. There were in these birds also many large tapeworms, *Davainea urogalli*, in the intestine, fragments of which were also rarely found in the caeca. In the birds examined during the spring there were invariably enormous numbers of the slender tapeworm, *Hymenolepis microps*, in the duodenum, and the mucous membrane of this part of the intestine was reddened.

It therefore remained to carefully compare normal and diseased birds (a) as to numbers of strongyli, (b) liability to contain living

bacilli in their organs, and (c) to make a detailed examination of the lesions in the caecal mucous membrane and to see what relation this had to the nematodes on the one hand and the bacilli on the other, and lastly (d) to find out whether or no the bacilli exerted any pathogenic action. It seemed possible that the strongyli might be the cause of the changes in the caecal mucous membrane: that these changes might admit the intestinal bacteria to the liver and other organs of the body, and that these together with other pathogenic products abnormally absorbed from the diseased caeca, or possibly the mere interference with absorption caused by that disease, might lead to the death of the birds.

All the diseased birds examined were considerably under weight and wasted. We never came across any instance of a bird dying plump and in good condition unless indeed its death could clearly be attributed to some other cause, such as having flown against a wall.

Methods of making cultures from the organs of birds.

It was recognized from the first that if micro-organisms were present only in small numbers in the organs somewhat large amounts of tissue might have to be used in order to obtain colonies on solid culture media. It was further recognized that the tissue would have to be crushed into a pulp, which could be spread more or less evenly over the surface of the medium, in order that any micro-organisms which might be present should have a chance of coming into contact with it. Moreover, it was clearly seen that in carrying out experiments of this kind the chances of accidental contamination were not inconsiderable. The methods which were first employed under rather difficult conditions in Scotland were later somewhat modified when the investigations were subsequently continued in Cambridge.

The precise conditions under which these experiments were conducted are matters of considerable importance, since upon them depend the reliability of the results which were obtained. We have therefore no hesitation in describing the methods in detail.

1. Precautions against aerial contamination.

Previous to beginning an experiment the room was carefully prepared. All dust was removed from the window ledges and elsewhere, and the floor and bench were flooded with a mixture of glycerine and lysol to lay the dust. All the windows and ventilation shafts were

closed during the actual operation of making the cultures. As a further precaution against aerial contamination the tissues were crushed inside a glass frame (Plate I, fig. 1). Two sheets of plate glass, 21 × 8 inches, formed the top and bottom respectively, the former being supported on blocks of wood, which formed the sides. The back was also formed of a sheet of plate glass, and the front was closed by a curtain of linen, which was soaked in lysol and could be partially turned back when required. The joints of the frame were made draught proof by means of rubber tubing. On the floor of the frame another sheet of plate glass, which extended the whole length, but was three inches narrower than the bottom, was placed to form a ledge near the centre of the floor, upon which the plates used for crushing the tissues could be conveniently manipulated, and yet be covered by the roof. The height of the frame from the top to this ledge was three and a half inches.

Before use the frame was washed out with a mixture of glycerine and lysol. In order to estimate the risk of aerial contamination agar plates were exposed on the bench and inside the frame during the whole period of time the cultures were being made.

2. *The preparation of the bird.*

The birds, if living, were killed by decapitation, weighed and immediately plucked in an adjoining room. As far as possible all the larger feathers, except those of the wings, were removed, and the cloaca, if gaping, was plugged with a pledget of cotton wool. The smaller feathers appeared to us to be a particularly dangerous source of contamination, since some might be soiled with faecal matter. Owing to their extreme lightness some of these unless carefully destroyed might float in the air and alight on to the tissues during the manipulations. The smallest feathers are only seen with difficulty, and might easily contaminate pieces of tissue as they were being removed from the body. In order to obviate all chance of contamination from feathers the body of the bird after plucking was held in the flame until all the minute feathers had been completely destroyed.

3. *The method of obtaining portions of the organs.*

A plumber's soldering iron, heated to redness, was freely used to burn the skin through which the incisions for removing pieces of tissue were to be made. The necessary incisions were then made without

delay with instruments sterilised by boiling for at least half-an-hour. A fresh pair of scissors and forceps were used for removing the piece of tissue actually used for cultivation. Cultivations were made from each organ in turn, observing the precautions which have just been described in each case. *Lungs*.—The lungs were approached from the back. After the skin had been thoroughly seared with the iron the muscles under the scapula were transfixed with a knife and the scapula freed by carrying the knife out to its apex; the bone was then turned up and broken. Next two or more ribs were cut through in two places, about half an inch apart, with scissors, and a piece of the lung approximately equal in bulk to a cube one-quarter of an inch in all dimensions was cut out, and quickly transferred to the ground glass plates for disintegration. *Kidneys*.—As the kidneys were approached from the back they were taken immediately after the lungs. A piece of the thin iliac bone, where it bulges outwards, was removed, care being taken not to force the intestines upwards during the process by pressure on the body. The satisfactory removal of portions of the kidneys was often a difficult matter, partly owing to the limited size of the opening which could be conveniently made in the bone, and partly owing to the nerve trunks which traverse the organs and render the extraction of portions difficult. In a few cases the intestine was wounded, but when this accident was perceived at once the attempt to obtain any further cultures from this bird was abandoned. *Liver, Pancreas and Spleen*.—These organs were approached from the front by turning the sternum back after cauterising the whole ventral surface, and especially the lines of the incision. Culture tubes were always sown from the liver, but the pancreas was only examined culturally on a few occasions, and cultures from the spleen were not made when the organ was required for histological purposes. In the grouse the spleen is extremely small, so that even when cultures were made the amount of material employed was considerably less than in the case of other organs. *Blood and Bile*.—Samples of blood were obtained by plunging sterile pipettes through the heart wall after cauterisation. Bile was also obtained in glass pipettes from the gall bladder, but the surface of the latter organ was not cauterised.

4. *The method of crushing the tissues.*

From each organ a piece, at least a quarter of an inch square, was removed by the methods just described, and placed on the surface of a ground glass plate. The plates used were 3 to 4 inches in diameter

and were ground on one side; these were sterilised by boiling and dried separately in the flame. As soon as they were dry the plates were placed in pairs, with their ground surfaces in contact on the glass ledge which has been described in the glass frame; in this situation they cooled rapidly. When a portion of an organ was ready to be ground up the upper plate of a pair was taken up and held in the fingers in such a way that about one-half or one-quarter of it overlapped an equal area of the lower plate. The piece of tissue was then placed between the overlapping areas and crushed. It was not found necessary to use powdered glass or other material to assist disintegration, because the organs of the bird, protected as they are from violence by the comparatively rigid skeleton, are much softer than those of mammals, and are easily reduced to the condition of an emulsion.

5. *The method of making cultures.*

Before starting an experiment a series of sloped agar tubes were labelled, two or three for each organ, and arranged on the bench in the order in which the organs were to be dealt with. As soon as a portion of an organ had been reduced to a pulp a considerable quantity of the pulp was taken up on a sterile platinum wire, bent into a series of loops so as to form a spatula, and spread over the surface of one of the agar tubes. The whole of the material crushed was left on the two tubes. In this way any living organisms that might be present had an opportunity of producing colonies on the surface of the medium. In the case of 9 birds (Nos. 20—28) anaerobic cultures in Buchner tubes were also made from all the organs, but as they did not yield anything more than the ordinary cultures, such cultures were not made in the later experiments.

6. *The examination of cultures.*

The cultures were incubated at 37° C. and examined daily on the first few days, and subsequently at various intervals up to a fortnight. Colonies of *B. coli* or *streptococci* seldom appeared after 24—48 hours' cultivation, except when they grew out of one of the larger masses of tissue on the surface of the tube. The principal result of allowing the cultures to incubate for longer periods was to reveal the presence of moulds and streptothrices, and occasional spore-bearing bacilli and cocci in cultures from the lungs.

The examination of the agar plates, exposed on the bench and within the glass frame during the progress of the experiments, showed that in spite of the long exposure very few colonies grew on them. *B. coli* was never found and moulds and streptothrices were uncommon. The commonest organisms were *S. lutea* and cocci.

7. The identification of the organisms found.

All organisms resembling *B. coli* were isolated in pure culture and the characters of their growth on agar, gelatin and potato together with their staining reactions and motility investigated. They were also cultivated up to 14 days in milk and in peptone water tubes containing glucose, lactose, mannite, saccharose, and dulcite.

Altogether 35 lactose fermenting organisms of the colon group were isolated from the organs of the birds and thoroughly investigated. All these organisms had the following characters in common:—Short, Gram negative, non-spore-producing bacilli with rounded ends. Greyish white colonies on agar, gelatin never liquefied. Moist white or cream coloured growth on potato. Permanent acidity and clotting in milk. Most of them produced indol, and the majority showed some motility, especially in peptone water cultures.

Following MacConkey's¹ (1905) classification of the lactose fermenting bacilli, these organisms may be divided, according to their reactions, which are given in the following table, into four groups.

Group	Type	Glucose	Lactose	Sac- charose	Dulcite	Mannite	Milk	Indol
I.	<i>B. acidi lactici</i> (Hüppe)	A + G	A + G	0	0	A + G	A + C	+
II.	<i>B. coli communis</i> (Escherich)	A + G	A + G	0	A + G	A + G	A + C	+
III.	<i>B. coli communior</i> (Durham)	A + G	A + G	A + G	A + G	A + G	A + C	+
IV.	<i>B. lactis aerogenes</i> (Escherich)	A + G	A + G	A + G	0	A + G	A + C	+

0 = No change.

A + C = Acid and clot.

A + G = Acid and gas produced.

+ = Positive reaction.

Twelve organisms, namely those isolated from the livers of grouse 3, 4, 7, 12, 44, 50, 56 and 60, from the lungs of 15, 50 and 57 and from the blood of grouse 15, belonged to group I; 3, isolated from the livers of grouse 11 and 53 and from the lungs of grouse 1, belonged to group II; 11, isolated from the livers of grouse 1, 6, 13, 16, 46, 51, 54, 58, 59 and 61 and from the lungs of grouse 13, belonged to group III; and 9 isolated

¹ MacConkey, A. (1905). "Lactose-fermenting bacteria in faeces," *Journ. of Hygiene*, v. p. 333.

from the livers of grouse 2, 22, 23, 57 and 62, from the lungs of grouse 22 and 56, and from the spleen and kidney of grouse 22, belonged to group IV.

Organisms belonging to all four groups were cultivated also from the caecal contents on various occasions.

An organism with the same general morphological and cultural characters, but differing in its fermentation reactions, was cultivated from the liver and spleen of grouse 47, and from both lungs, spleen, pancreas and both kidneys of grouse 51. This organism produces acid and gas in media containing glucose, mannite and dulcitol, and acidity followed by alkalinity in milk. In media containing lactose and saccharose no change is produced. It corresponds therefore in its cultural characters with the *B. enteritidis* (Gaertner) group.

Many of the other organisms found were similarly investigated except moulds, streptothrices, cocci and spore-bearing bacilli, but in view of their extreme rarity it seems scarcely necessary to give their cultural characters in detail.

The method of counting strongyli in the caeca.

At an early stage in the investigations it began to appear probable that the presence of *B. coli* in the liver and other organs of the grouse was related in some way to the numbers of *Trichostrongylus pergracilis* in the caeca. Up to December 1908 the strongyli were only roughly estimated, but at that time a method of isolating and counting them was devised and found to be practicable, and from that time onwards the strongyli were counted in every case. The method was as follows: The caeca were laid out straight on a board and opened throughout their length, their contents turned out and their mucous membrane scraped. All the material liable to contain strongyli was thus collected. Little by little this was shaken up with water in a large test-tube and poured out drop by drop into a Petri dish containing water. With suitable illumination the strongyli could be clearly seen and picked out with a mounted needle and counted. When the contents of the caeca were drier than usual, and did not readily break up when shaken with water, they were disintegrated by rubbing between the flat surface of a rubber bung and the bottom of a Petri dish. There can be no doubt that, while some strongyli must have escaped notice, this method gave a close approximation to the numbers which were actually present, quite close enough for the purposes of our inquiry. In nearly all cases the worms

in the two caeca were separately counted, usually by different observers. As may be seen, by reference to Table I and Plate II, fig. 3, in all but two birds (56 and 67) approximately equal numbers were present in the two caeca. We thought therefore that in our future investigations a sufficiently accurate estimation of the number of strongyli might be arrived at by counting those present in one caecum and doubling the number found.

TABLE I.

Showing the results of counting the strongyli in the two caeca separately.

Grouse No.	Strongyli		Total	
	One caecum	Other caecum		
52	0	0	0	23 specimens of <i>Heterakis papillosa</i> found in one caecum and 10 in the other.
81	0	0	0	
58	54	59	113	
65	89	94	183	1 specimen of <i>H. papillosa</i> in each caecum.
59	108	127	235	
46	131	128	259	
55	201	214	415	
63	281	252	533	
64	268	303	571	
57	331	268	599	
62	365	375	730	
67	285	548	833	
68	420	457	877	
66	455	490	945	
56	754	1114	1868	
53	1103	1403	2506	1 specimen of <i>H. papillosa</i> in each caecum.
60	3118	2877	5995	
61	4769	4793	9562	

General results of bacteriological examinations of the organs.

In the lungs, moulds and streptothrices were almost constantly found. The fact that they were absent in all but a very few of the tubes sown from other organs indicates that they were really in the birds' lungs during life, and did not get into the tubes as a result of contamination. Further, these results have been confirmed by observations on a number of other animals, both mammals and birds.

The other organs and blood were in the immense majority of cases free from cultivatable micro-organisms, except when *B. coli* was present. Occasionally a single colony of some microbe would appear, perhaps a spore-bearing bacillus like *B. subtilis*, or *Sarcina lutea*, or rarely a mould.

On several occasions diphtheroid segmented bacilli were found. That these were sometimes accidental contaminations seems very probable, and in any case their numbers were so few as to be of little practical importance. Nevertheless, it may be that some were really in the living tissues during life, and this seems more probable in the case of the segmented bacilli, which were sometimes found in cultures from the blood, which are less liable to contamination than those from the solid organs, as well as elsewhere. Moreover in one case (grouse 37) they were also cultivated from the contents of the intestinal canal, but were only rarely met with on the exposed agar plates.

The whole question of the presence of bacteria in the living organs is in the course of investigation by us, and we need not dwell further on the matter here, except in so far as *B. coli* is concerned.

Note on the alimentary canal of the normal grouse¹.

Beyond the oesophagus, crop and gizzard the alimentary canal consists of the duodenum, intestine, paired caeca and rectum (Plate I, fig. 2).

The duodenum, a thin-walled light-coloured tube, four to seven inches long, on which the vessels are clearly seen, forms a U-shaped loop, of which the limbs lie in close contact with one another, the angular space on the ventral side being occupied by the pancreas. Next follows the intestine, a thicker walled tube of grey colour some 20 to 34 inches in length, and half an inch in diameter. From the junction of the intestine and rectum arise the paired caeca. Each caecum consists of a short narrow portion with small lumen next the intestine, and a long wider portion between one and two feet, or even more, in length, and about one-third of an inch in diameter. At its distal end it tapers rather suddenly to a point. Its walls are thinner than those of the intestine and are marked by about nine longitudinal whitish lines. On opening the caecum well marked longitudinal ridges are seen, corresponding to the lines just described. Each ridge shows alternate thicker and thinner portions. Occasionally one of the ridges may be seen to die away or fuse with its neighbour. They occur throughout the whole length of the caecum.

On examination under a Zeiss binocular microscope ($\times 8-33$) the mucous membrane (of a grouse (No. 81) in which no strongyli are

¹ The measurements of the various parts of the alimentary canal vary greatly in different birds.

present), after gentle washing in a stream of water, is seen to be regularly beset with small villi of uniform size, arranged closely together on the ridges, but more widely separated in the depressions, where they seem to be less well developed. They often appear club-shaped, more especially on the ridges, where their flattened terminations, lying closely together at a uniform level, give the surface a somewhat smooth and tessellated appearance (Plate IV, fig. 11). In birds (e.g. No. 69) caught on the moor and apparently normal but infected with strongyli both the ridges and the villi are much larger (Plate IV, fig. 12).

The rectum is a thick walled tube of greyish white colour, about four inches in length.

The contents of the gut vary much in different parts. The duodenum usually contains nothing but a white slimy mucus. The intestine contains coarsely divided particles of food and occasional stones from the gizzard. The contents of the caeca present a marked contrast to those of the intestine, consisting of a brownish or greenish pasty mass of finely divided material. The rectum contains usually only the coarser particles of food which have never passed into the caeca.

Pathological changes in the alimentary canal.

Duodenum. At certain seasons of the year the duodenum of every wild bird examined was packed with the long tape worm *Hymenolepis microps*. They were particularly numerous from March to May and towards the end of August. Under these circumstances the contents appear to consist wholly of tenacious mucus, until shaken up in alcohol, when the worm becomes visible for the first time. No obvious pathological changes, except some reddening, were seen. *Trichosoma longicolle* was occasionally found in small numbers.

Intestine. The lower half of the intestine was often found distended with tangled masses of the large tape-worm, *Davainea urogalli*; they bear a less definite relation to season than does *Hymenolepis*. Portions of these worms are sometimes found bile stained. No pathological changes were noticed.

Caeca. The appearance of the caecum as seen from without varied; in some cases there were no obvious changes; in some cases the caeca appeared to be somewhat dilated, in others they appeared mottled with lighter coloured patches. The contents, the main portion of which was semi-fluid, often contained—especially near the proximal ends—dry masses, which were very adherent to the mucous membrane, and which

corresponded to the whitish patches seen from the exterior. Whenever one of these masses was peeled off numerous strongyli could be seen stretched between the mass and the mucous membrane, obviously adherent to both. The dry attached condition of these masses strongly suggested that they represented material which had long been retained in the gut.

The small nematode, *Trichostrongylus pergracilis*, was often present in enormous numbers, occasionally amounting to thousands. They were particularly numerous towards the proximal ends of the caeca, especially in the dry masses just described. Except in one instance we never failed to find strongyli in wild grouse, and they were always present in large numbers in birds suffering from grouse disease. The numbers present in wild grouse did not appear to depend in any way upon the time of year. Portions of *Davainea* were on rare occasions seen in the caeca.

After washing in a gentle stream of water the mucous membrane frequently appeared reddened, especially in birds which were picked up dead on the moor. The reddening was present in many, but not in all, of the birds badly infested with strongyli which were examined in a perfectly fresh condition. It was thought that this might possibly have been a post-mortem change, and some normal birds were kept after death for a few days before examination to see if the redness would appear in them, but it was not seen. When examined under a Zeiss binocular microscope ($\times 8-33$) the ridges were found to be thickened, especially in patches, to which the dry masses already referred to were found adherent (Plate IV, fig. 13). The villi were very irregular in all situations, being in places greatly hypertrophied and club-shaped both in the depressions and on the ridges, and in other places atrophied, particularly on the thickenings just mentioned. In many cases the villi on the ridges were embedded in some cementing material, which in microscopic sections appeared to be composed of a mixture of mucous and granular debris, which could not be removed by gentle washing. Even after free washing numerous strongyli could be seen adherent to the mucous membrane, and frequently penetrating between the villi (Plate V, fig. 17). In some of the worst cases the ridges are so deformed as to resemble masses of coral, with smooth but irregular surfaces, on which the individual villi are frequently indistinguishable, and with cave-like depressions between them from which one or more strongyli can be seen protruding (Plate IV, fig. 14). These appearances, we believe, are due to the matting together of the villi and

sometimes of the neighbouring ridges by the cementing material described above.

Histological changes in the caeca.

With the small amount of material at our disposal¹ it was impossible to follow out in detail the various changes which occur in the caeca, and we therefore confine ourselves to comparing the condition found in severely affected birds with that found in normal birds. In the investigation of the histological changes we had the advantage of the expert opinion of Mr T. S. P. Strangeways, Huddersfield Lecturer in Special Pathology, Cambridge, to whom we are greatly indebted.

Sections of the caecum of the normal bird (No. 81) without strongyli show the following structures.

There is under the peritoneum a well marked muscular coat, and within this delicate areolar tissue supporting a layer of well formed connective tissue on which the mucous membrane rests. At intervals the connective tissue layer projects towards the lumen of the gut forming the central core of the ridges which have been described. At their bases these prolongations appear bifurcated, and the spaces between the bifurcations are filled with fat and some large blood vessels. Both the ridges and the depressions between them are covered with villi of fairly uniform length, which consist of a central core of vessels surrounded by a small quantity of delicate sub-epithelial connective tissue, together with a few lymphoid cells, covered with a single layer of columnar epithelium. Here and there in the depressions may be seen sections of lymphoid follicles covered with villi. The contents lying in the lumen of the gut consist of a mass of granular material and mucus (Plate II, figs. 5 and 6).

Sections of the caecum of an apparently healthy grouse (No. 69) with many strongyli (1460) caught on the moor differ in certain respects (Plate III, fig. 7). The muscular walls contain distinct bands of wavy fibrous tissue. The quantity of fibrous tissue in the cores of the ridges seems to be increased; but fat is still present in the bifurcations. The ridges are large and the villi are markedly increased in size, especially those situated near the free margins of the ridges. In the latter wavy bands of fibrous tissue may be seen; and lymphoid cells are found in considerable numbers within all the villi. The epithelium appears hypertrophied, but is not markedly irregular except over the villi on the free

¹ In 26 specimens the contents of both caeca were used for counting the strongyli; and 14 specimens arrived dead and therefore useless for minute histological examination.

margins of the ridges. Worms are uncommon except in certain situations in the depressions, where they seem to be entangled in what appears to be dry, concentrated gut contents. No lymphoid follicles can be seen.

In a diseased grouse (No. 6), in which the macroscopic changes are well marked, the following condition is found. The muscular wall contains well marked strands of fibrous tissue. The fat at the bases of the ridges has completely disappeared, and the vessels show considerable thickening of their walls. The connective tissue in the cores of the ridges is also greatly increased in amount and in density and the vessels dilated. The sub-epithelial connective tissue of the villi is also increased in amount and the vessels in it dilated, and probably increased in number, and in some cases full of blood. The connective tissue is in most places loose and contains large numbers of cells, probably inflammatory in origin, and in some places, especially near the free ends of the villi and in the neighbourhood of the worms, shows fibroid change. The epithelium is proliferated and thrown into folds (Plate III, fig. 8).

In a grouse (No. 15) badly infected with strongyli and showing well marked macroscopic lesions all the changes just described are more evident. Much fibrous tissue is present in the muscular coat, and the walls of the vessels are very markedly thickened. The villi appear increased in size, and their connective tissue is more dense, and contains a considerable amount of fibrous tissue, replacing the more delicate connective tissue. In this tissue a large number of the nuclei are clearly those of newly formed fibrous tissue, being elongated and spindle-shaped, though round cells are still present in considerable numbers. Nuclei of the former type are now found in all situations, and are not limited to the cores of the ridges as in the case of specimens from normal birds. The epithelium shows great proliferative changes and is thrown into irregular folds. In all specimens from diseased birds the lymphoid follicles are indistinguishable (Plate III, figs. 9 and 10).

In fact, the general condition shows evidence of a chronic inflammation leading to fibrosis. Large quantities of mucus are present in the intestinal contents, and the villi appear to be united together with this material, which penetrates to the deepest parts of the crypts between the villi. Everywhere strongyli are present, and their relationship to the structures composing the wall of the organ is of special interest (Plate III, fig. 10). They are found in large numbers both in the lumen and between the villi, in some instances having penetrated to the deepest portions of the crypts. In such cases the epithelium covering the portions of the villi adjacent to the worms is greatly altered, and a

marked increase of fibrous tissue in the underlying connective tissue is frequently observed (Plate V, fig. 18). In some instances the epithelium has completely disappeared all round the worm so that the latter is seen surrounded by a ring of dense fibrous tissue (Plate IV, fig. 16). Occasionally a worm is found lying between the epithelium and the matrix of the villus, which usually shows fibroid change in the neighbourhood (Plate IV, fig. 15).

There can be little doubt therefore that the presence of the worms in such situations leads to chronic inflammatory changes and fibrosis.

The relation of B. coli in the organs to strongyli in the caeca.

As has already been stated it began to appear probable early in the course of the work that the presence of *B. coli* in the liver and other organs was related in some way to the numbers of strongyli in the caeca. In several birds which had been raised in captivity in Scotland and subsequently kept in Surrey no strongyli could be found, even after a careful search, and in their organs there were no bacilli of the colon type (one exception). On the other hand, in the organs of grouse with very large numbers of strongyli *B. coli* was constantly present, either in the liver or in some other organ. In other grouse with fewer strongyli *B. coli* was present in some and appeared to be absent in others.

The results obtained previous to the adoption of the counting method are shown in Table II, p. 19, in which the birds are arranged in three classes.

In the birds of class I *B. coli* was found once only in the organs, and then in a grouse with very numerous tape worms in the duodenum and intestine, and numerous portions of *Davainea* in the caeca, a thing very rarely observed and probably indicating some abnormal condition. *B. coli* was found in the liver of one of six birds belonging to class II. Amongst the birds belonging to class III *B. coli* was constantly found in the liver (one exception). In five instances they were cultivated from one of the other organs also.

Since the counting of the strongyli was systematically undertaken 23 presumably healthy birds have been examined. The results confirmed the opinions previously arrived at. In this series four birds had less than 100 strongyli, and *B. coli* was not found in their organs. Fifteen had strongyli varying in number between 100 and 1000, and *B. coli* was cultivated from the organs of some (8) and not from those of

TABLE II.

Showing the results of cultures previous to the adoption of the method of counting strongyli.

	Grouse No.	Intestinal worms			Cultures from the organs ¹			
		<i>Hymenolæpis</i>	<i>Davainea</i>	<i>Strongylus</i>	Liver	Lungs	Spleen	Kidney
Class I. No strongyli. No <i>B. coli</i> .	5.	0	0	—	0	0	0	—
	18.	0	0	0	0	0	0	0
	19.	0	0	0	0	0	0	0
	21.	0	0	0	0	0	0	0
	23.	Numerous	Numerous	0	<i>B. coli</i>	0	<i>B. coli</i>	0*
	25.	0	0	0	0	0	0	0
	26.	0	One	0	0	0	0	0
Class II. ² Few strongyli. <i>B. coli</i> inconsistent.	16.	0	0	Few	—	0	—	—
	17.	Few	One	Eggs only	0	0	0	0
	30.	Few	Numerous	Few	0	0	0	0
	33.	Moderate	Numerous	Few	<i>B. coli</i>	0	0	0
	34.	Few	Moderate	Few	0	0	0	0
	40.	0	0	Few	0	0	0	—
Class III. Many strongyli. <i>B. coli</i> constant.	1.	Numerous	—	Numerous	<i>B. coli</i>	<i>B. coli</i>	—	—
	2.	Numerous	Numerous	Numerous	<i>B. coli</i>	0	—	—
	3.	Numerous	Few	Numerous	<i>B. coli</i>	0	—	—
	4.	Numerous	Numerous	Numerous	<i>B. coli</i>	0	—	—
	6.	Numerous	Numerous	Numerous	<i>B. coli</i>	0	—	—
	11.	Numerous	Numerous	Numerous	<i>B. coli</i>	0	—	—
	12.	Numerous	Numerous	Numerous	<i>B. coli</i>	0	—	—
	13.	Numerous	0	Numerous	<i>B. coli</i>	<i>B. coli</i>	—	—
	14.	Numerous	0	Numerous	<i>B. coli</i>	0	—	—
	15.	Numerous	Numerous	Numerous	<i>B. coli</i>	<i>B. coli</i>	—	—
	22.	0	Two	Numerous	<i>B. coli</i>	0	0	<i>B. coli</i>
	28.	0	0	Numerous	<i>B. coli</i>	0	0	0
	29.	0	0	Numerous	<i>B. coli</i>	0	0	0
	31.	Numerous	Few	Numerous	0	0	<i>B. coli</i>	0
	32.	Moderate	Moderate	Numerous	<i>B. coli</i>	0	0	0
	35.	Numerous	Numerous	Numerous	<i>B. coli</i>	0	0	0

* Numerous portions of *Davainea* in the caeca.

¹ In this and the following Table 0 indicates that no organisms of the *B. coli* type were present in the cultures, and — that cultures were not made.

² In the birds of Class II the strongyli were noted as few, but subsequent experience with counting methods showed that what appeared few to an ordinary examination might sometimes turn out to be 100 or more when counted.

others (7). In four birds in which very large numbers of strongyli, *i.e.* over 1000, were counted *B. coli* was found in the livers of all. The results of these observations are given in the following table.

TABLE III.

Showing the results of cultures after the adoption of the method of counting strongyli.

Grouse No.	Number of strongyli	Cultures from organs			
		Liver	Lungs	Spleen	Kidneys
52	0	0	0	0	0
81	0	0	0	0	0
48	32	0	0	0	0
43	45	0	0	0	0
58	113	0	0	0	0
59	235	<i>B. coli</i>	0	0	0
46	259	<i>B. coli</i>	0	0	0
50	290	<i>B. coli</i>	<i>B. coli</i>	<i>B. coli</i>	0
49	330	0	0	0	0
47	344	<i>B. ent.</i>	0	<i>B. ent.</i>	0
55	415	0	0	0	0
63	533	0	0	0	0
51	540	<i>B. coli</i>	<i>B. ent.</i>	<i>B. ent.</i>	<i>B. ent.</i>
64	571	0	0	0	0
57	599	<i>B. coli</i>	0	0	0
62	730	<i>B. coli</i>	0	0	0
67	833	0	0	0	0
44	871	<i>B. coli</i>	0	0	0
66	945	0	0	0	0
54	1645	<i>B. coli</i>	0	<i>B. coli</i>	0
56	1868	<i>B. coli</i>	<i>B. coli</i>	0	—
60	5995	<i>B. coli</i>	0	0	0
61	9562	<i>B. coli</i>	0	0	0

B. ent. = *B. enteritidis*.

The points which come out clearly from these two tables are:— (1) that when strongyli are absent from the caeca or are present only in small numbers (less than 100) intestinal bacteria, especially *B. coli*, are not present in the liver or other organs of the grouse (11 grouse—1 exception); (2) that when relatively few strongyli are present (100—1000) *B. coli* may or may not be present in the organs (21 grouse); and (3) that when enormous numbers (over 1000) are present *B. coli* has invariably been found in the liver or other organs (20 grouse).

It has not been found possible to estimate the numbers of living *B. coli* present from the number of colonies which grew on the tubes.

Sometimes of course we were able to make a rough guess. In some control birds both diseased and healthy, which were examined some considerable time after death, the innumerable number of colonies on the tubes showed that *B. coli* was at that time swarming in the tissues. But with living birds, even when very large numbers of strongyli were present, the colonies of *B. coli* were few in number, one or two to ten or a dozen, and rarely more than thirty.

It is not claimed, of course, that the number of living *B. coli*, in the liver for example, is exactly proportional to the number of strongyli. With as few strongyli as 235, *B. coli* has been found (one colony in one tube), and again with as many as 945, *B. coli* has been absent. Doubtless other conditions which affect the health of the bird influence the permeability of the intestinal wall to the contained bacteria.

The numbers of strongyli present in healthy and diseased birds.

We have already shown that strongyli are almost constantly present in the caeca of wild grouse believed to be perfectly normal, and certainly of fair weight and in good general condition. In a few so-called healthy birds they may be present literally in thousands. We were informed by Dr Wilson that strongyli are more numerous in diseased than in healthy birds; and we have ourselves examined a number of diseased birds brought in dead, and useless for cultural purposes, and collected the worms from them.

Table IV shows that the number of strongyli present in diseased birds, though varying considerably, is greatly in excess of that found in the great majority of normal birds. In a small minority of the presumably healthy birds the numbers were as large as those found in many of the diseased birds. It is, of course, impossible to be certain that these exceptional birds were not really suffering from the early stages of grouse disease. The two (60, 61) with the largest numbers came from a moor on which grouse disease was prevalent at the time.

The presence in diseased birds of strongyli in numbers far in excess of those found in normal birds does not of course prove that they were the cause of the disease, because it is conceivable that they may have multiplied as a consequence of the disease. Nevertheless, taken in conjunction with the changes previously described in the mucous membrane of the caecum and the relation of the worms thereto, it is exceedingly probable that the worms are really the cause of the disease.

TABLE IV.

Showing the relative number of strongyli in healthy birds and those believed to be suffering from grouse disease.

Birds received alive, apparently in good health, or sent as average specimens of normal grouse		Diseased birds picked up dead	
Grouse No.	Number of strongyli	Grouse No.	Number of strongyli
81	0	53	2506
52	0	79	2556 (1278 in one caecum)
48	32	74	3114 (1557 „)
43	45	78	3340 (1670 „)
58	113	74 (a)	3406 (1703 „)
59	235	80	3840 (1920 „)
46	259	75	4352 (2176 „)
50	290	39	6230
49	330	71	7058 (3529 in one caecum)
47	344	73	7484 (3742 „)
55	415	77	8800 (4400 „)
63	533	72	10266 (5133 „)
51	540	76	18332 (9166 „)
64	571		
57	599		
62	730		
69	730*		
67	833		
44	871		
66	945		
54	1645		
56	1868		
70	2524*		
60	5995†		
61	9562†		

* One caecum only counted and the numbers doubled.

† These birds came from the same moor.

The relation of B. coli in the organs to tape-worms in the intestine.

The question arises whether the tape-worms, often present in enormous numbers (Plate V, figs. 19, 20) in the gut of the grouse, act like the strongylus and increase the permeability of the intestinal wall to bacteria. Tables V and VI show that there is little or no relation between the presence of tape-worms in the gut and *B. coli* in the organs. Numerous tape-worms of either kind, *Hymenolepis* and *Davainea*, might be present without any *B. coli* appearing in cultures from the liver; and on the other hand, *B. coli* might be present in the liver and yet one or other or both of the tape-worms might be absent.

TABLE V.

Showing that the presence of Hymenolepis in the duodenum is not related to the presence of B. coli in the liver.

Grouse No.	<i>Hymenolepis</i>	Cultures from the organs			
		Liver	Lungs	Spleen	Kidneys
52	0	0	0	0	0
81	0	0	0	0	0
48	0	0	0	0	0
43	0	0	0	0	0
49	0	0	0	0	0
58	0	0	0	0	0
55	0	0	0	0	0
59	0	<i>B. coli</i>	0	0	0
46	0	<i>B. coli</i>	0	0	0
50	0	<i>B. coli</i>	<i>B. coli</i>	<i>B. coli</i>	0
47	0	<i>B. ent.</i>	0	<i>B. ent.</i>	0
51	0	<i>B. coli</i>	<i>B. ent.</i>	<i>B. ent.</i>	<i>B. ent.</i>
57	0	<i>B. coli</i>	0	0	0
62	0	<i>B. coli</i>	0	0	0
44	0	<i>B. coli</i>	0	0	0
56	0	<i>B. coli</i>	<i>B. coli</i>	0	0
54	0	<i>B. coli</i>	0	<i>B. coli</i>	0
60	Few	<i>B. coli</i>	0	0	0
63	Numerous	0	0	0	0
64	Numerous	0	0	0	0
67	Numerous	0	0	0	0
66	Numerous	0	0	0	0
61	Numerous	<i>B. coli</i>	0	0	0

B. ent. = *B. enteritidis*.

The prevalence of Hymenolepis and Davainea in normal and diseased birds.

It is not without significance that *Hymenolepis*, so numerous from the spring to the autumn months, during which the greatest mortality takes place, is scarce or absent during the winter, when the disease is quiescent. The enormous numbers which both these worms may attain is almost unbelievable by one who has not seen them (Plate V, figs. 19, 20). *Davainea* seems to be present in the intestine throughout the year. On the other hand, we have not observed any gross lesions in the neighbouring mucous membrane even in the worst cases of infection with either of these worms, nor have they, as has already been shown, any

relation to the presence of living intestinal bacteria in the tissues. We therefore are not inclined to believe that they play any part, except perhaps a secondary one, in the causation of grouse disease.

TABLE VI.

Showing that the presence of Davainea in the intestine is not related to the presence of B. coli in the liver.

Grouse No.	Davainea	Cultures from the organs			
		Liver	Lungs	Spleen	Kidneys
52	0	0	0	0	0
81	0	0	0	0	0
48	0	0	0	0	0
58	0	0	0	0	0
59	0	<i>B. coli</i>	0	0	0
46	0	<i>B. coli</i>	0	0	0
50	0	<i>B. coli</i>	<i>B. coli</i>	<i>B. coli</i>	0
63	0	0	0	0	0
51	0	<i>B. coli</i>	<i>B. ent.</i>	<i>B. ent.</i>	<i>B. ent.</i>
62	0	<i>B. coli</i>	0	0	0
44	0	<i>B. coli</i>	0	0	0
43	One	0	0	0	0
66	One	0	0	0	0
56	Five	<i>B. coli</i>	<i>B. coli</i>	0	0
54	Six	<i>B. coli</i>	0	<i>B. coli</i>	0
47	Seven	<i>B. ent.</i>	0	<i>B. ent.</i>	0
55	Seven	0	0	0	0
57	Eleven	<i>B. coli</i>	0	0	0
67	Moderate	0	0	0	0
49	Moderate	0	0	0	0
64	Numerous	0	0	0	0
60	Numerous	<i>B. coli</i>	0	0	0
61	Numerous	<i>B. coli</i>	0	0	0

B. ent. = *B. enteritidis*.

In the above tables the birds are arranged in order, according to the numbers of tape-worms present. When two or more birds had the same number of these worms they are arranged according to the number of strongyli.

The relation of B. coli in the liver to coccidiosis of the intestine.

Three grouse chicks, reared at Frimley and experimentally fed on coccidia by Dr Wilson, were examined by cultures for the presence of intestinal organisms in their organs.

The first (B. 15. Hatched 28. VI. 09. Faeces examined and no spores of coccidia found. Fed twice on 9. VII. and 17. VII with faeces

from other birds containing spores of coccidia. Killed 6. VIII.) was very ill and extremely emaciated when received, and was killed and examined immediately. An organism of the *B. enteritidis* type was found in the liver, but not in the other organs. No worms of any kind were found in the intestine or caeca. Sections of the gut examined by Dr Fantham showed numerous coccidia in all stages of multiplication in the cells. The second chick (B. 2. Hatched at the same time and treated in the same way) was also ill when received, and was killed and examined immediately. In this case a few colonies of *B. coli* were obtained from the liver cultures. No worms were found, and the condition of the intestine was the same as in the first chick. A third older chick (4 months) which had been fed on coccidia three weeks previously was also killed and examined. A few streptothrices developed on the cultures from the lungs, but those from the other organs remained sterile. Neither worms nor coccidia were found in the intestine or caeca.

These observations seem to indicate that intestinal coccidiosis may so injure the gut that bacteria are allowed to pass into the circulation. This conclusion is supported by eight observations on young rabbits suffering from naturally acquired coccidiosis of the intestine, the results of which are given in the following table.

TABLE VII.

Showing the results of cultures from the organs of young rabbits suffering from coccidiosis.

Rabbit No.			Cultures from the organs						Mesenteric gland	
	Coccidiosis		Liver	Spleen	Lungs	Kidneys	Blood	Bile		
	Intestine	Liver								
1.	No lesions	Well marked	0	0	0	0	0	0	<i>B. coli.</i>	No nematodes.
2.	„	Excessive	0	0	0	0	—	—	„	„
3.	„	Well marked	0	0	0	0	—	—	„	
4.	Trace only	Few small spots	0	0	0	0	0	—	„	Many nematodes.
5.	„	„	0	0	0	0	0	—	„	„ (233).
6.	Well marked	„	<i>B. coli</i>	0	0	0	—	—	„	„ (246).
7.	„	One spot	<i>B. enteritidis</i>	<i>B. enteritidis</i>	0	0	—		{ <i>B. coli</i> <i>B. enteritidis.</i>	No nematodes.
8.	„	Well marked	<i>B. coli</i>	0	0	0	0	—	<i>E. coli.</i>	

The mesenteric glands yielded intestinal bacteria in all cases. The cultures from the other organs, including the liver, yielded no intestinal bacteria when the small intestine was normal, or showed merely a trace of coccidiosis. On the other hand, when the small intestine showed well marked coccidiosis *B. coli* or *B. enteritidis* was always present in the liver, and sometimes in the other organs. The existence of coccidiosis of the liver bore no relation to the presence of *B. coli* in that organ, even the affected bile ducts being sterile. The presence of the nematode, *Oxyuris ambigua* (in moderate numbers) did not appear to have any influence on the passage of bacteria from the intestine into the blood vessels.

The significance of B. coli in the organs.

We have shown that *B. coli* is constantly present in the organs of birds whose caeca contain large numbers of strongyli, and we have shown that the latter are present in far larger numbers in diseased than in healthy birds. It may therefore be assumed that *B. coli*, while not invariably absent from the organs of the healthy bird, is constantly present in those of diseased birds. The small numbers of colonies of *B. coli* cultivated from the tissues of diseased grouse indicate that these bacteria do not multiply in the tissues. We therefore do not suggest that grouse disease is essentially an infection with these bacteria. But the number of colonies which appear on our tubes does not allow us to estimate even approximately the numbers of bacilli which enter the tissues and get killed. The products of these bacilli, if really numerous, doubtless exert some amount of harmful influence, but how much we are not at present in a position to say. No bacilli could with certainty be identified either in sections or in smears of the livers of diseased or healthy birds. Many of the cells were filled with large numbers of iron-containing granules, which seemed to be more numerous in diseased than in healthy birds. But the fact that we have not found either by macroscopic or microscopic examination any important lesions in the livers which have yielded cultures inclines us to think that the bacilli play only a secondary part in the causation of death.

Seasonal prevalence of the principal grouse entozoa.

Though this subject is being very fully dealt with by other workers for the Grouse Disease Inquiry we feel that it is desirable, in view of the statements we have made, to record our own observations in tabular

TABLE VIII.

Showing the seasonal prevalence of the principal grouse entozoa.

Apparently Healthy Birds.					Diseased birds.			
Hand-reared, shot, or caught by keepers as examples of healthy birds					Picked up dead on the moors or caught in a weak condition			
Date	Number	<i>Hymenolepis</i>	<i>Davainea</i>	<i>Strongylus</i>	Number	<i>Hymenolepis</i>	<i>Davainea</i>	<i>Strongylus</i>
Feb. 3, 1909	54	0	1	1645
"	55	0	7	415
" 5	56	0	5	1868
"	57	0	11	599
"	58	0	0	113
" 6	59	0	0	235
Mar. 17	60	Few	Numerous	5995
"	61	Numerous	"	9562
" 19	62	0	0	730
" 20	63	Numerous	0	533
"	64	"	Many	571
"	65	"	2	183
"	66	"	1	945
" 30	67	"	Moderate	833
April 22	68	Few	0	877
" 27	69	Numerous	Numerous	730*
"	70	"	"	2524*
May 10	71	Numerous	0	7058*
"	72	"	Numerous	10266*
" 5, 08	1	"	-	Numerous
"	2	"	Numerous	"
"	3	"	Few	"
"	4	"	Numerous	"
" 7, 08	5	0	0	-	6	"	"	"
" 7, 09	73	"	Fragments	7484*
" 9, 08	11	"	Numerous	Numerous
"	12	"	"	"
"	13	"	0	"
"	14	"	0	"
"	15	"	Numerous	"
" 19, 09	74	0	Moderate	3114*
"	74(a)	Numerous	0	3406*
"	75	"	Numerous	4352*
"	76	0	"	18332*
"	77	Numerous	0	8800*
" 24, 09	78	"	Few	3340*
June 3, 09	16	0	0	Few	79	Few	0	2556*
"	80	0	0	3840*
July 10, 08	17	Few	1	Few
"	18	0	0	0
"	19	0	0	0
" 28	20	0	0	Few
Aug. 5, 08	21	0	0	0
" 10	22	0	2	Numerous
" 15	23	Numerous	Numerous	0
" 18	25	0	0	0
"	26	0	1	0
" 26	28	0	0	Numerous	29	0	0	Numerous
" 30	30	Few	Numerous	Few
"	31	Numerous	Few	Numerous
" 30	32	Moderate	Moderate	Moderate
"	33	Numerous	Numerous	Few
"	34	Few	Moderate	"	35	Numerous	Numerous	Numerous
Sept. 2, 08	36	0	0	0
" 14	37	0	0	0
Oct. 23, 08	38	0	Numerous	Numerous	39	Few	Many	6230
" 28	40	0	0	Few	41	0	Moderate	Numerous
Nov. 8, 08	43	0	1	45
"	44	0	0	871	45	0	Moderate	Numerous
" 9	46	0	0	259
"	47	0	0	344
"	48	0	0	32
"	49	0	Several	330
"	50	0	0	290
"	51	0	0	540
" 17, 09	81	0	0	0
" 21, 08	52	0	0	0

* The strongyli in one caecum counted and number found doubled.

form. Though unfortunately we seldom had the opportunity of making observations on diseased and healthy birds at the same time the table shows that *Hymenolepis microps* occurred in large numbers in the great majority of birds examined from the middle of March to the end of May. Very few were present in the 13 birds examined between the beginning of June and the last week in August. During the last few days in August they were met with in moderate numbers in all the birds examined. From the beginning of September to the beginning of February they were absent from all the birds examined with one exception¹. The relation to season is much less marked in the case of *Davainea urogalli*, though it occurred in the greatest numbers at the same seasons as *Hymenolepis*.

With regard to *Trichostrongylus pergracilis* it is difficult to come to any definite conclusion as to its seasonal prevalence from our own observations, conducted as they were on diseased birds at one time of year, and on healthy, often hand-reared, birds at another; but it is clear that they do not disappear at any season.

Summary.

The causes of death of the grouse are, of course, various. We ourselves have seen pleuropneumonia (in a bird long kept in captivity in Cambridge), pericarditis, necrotic patches in the liver, an obscure chronic disease of the peritoneum, and septic infection from a gangrenous fracture of the wing. On the other hand the great majority of birds, either picked up dead on the moor, or caught by keepers when weak and unable to fly, have been found to be all more or less in the same condition; they were wasted, badly infested with *Trichostrongylus pergracilis*, and often also with *Davainea urogalli* or *Hymenolepis microps*, or with both. More or less pathological change was seen in the caeca; the mucous membrane was often reddened, and under the binocular microscope considerable changes were seen, though we did not observe gross ulceration. Sections examined under the higher powers showed serious chronic inflammatory changes particularly in the immediate neighbourhood of the worms.

¹ Our observations have been made on a small number of birds, but Dr E. A. Wilson, who will shortly publish statistics based on the examination of a very large number of birds during several years, informs us that *Davainea* is abundant throughout the year, and that *Hymenolepis* is abundant from May to October. The numbers of the latter gradually diminish from November to February, but rise suddenly in the early part of March.

Birds showing these changes we take to be representative of the chronic form of grouse disease. Whether there be also an acute epizootic disease among grouse we cannot tell. We can only say that, so far as our experience goes, we have not seen it. We have never seen pneumonia in the wild bird, and we have never seen any birds picked up dead when plump and in good condition without finding evidence that they had died of injury.

We have therefore to discuss the causes of death in the chronic wasting disease, which is observed among grouse fairly regularly in the Spring and to a lesser extent in the Autumn, and it is to this we refer when we speak of "grouse disease."

First we must consider the gross intestinal parasites which occur in such remarkable numbers in the grouse. The tape-worm *Hymenolepis microps* alone shows any relation in its seasonal prevalence to grouse disease. This worm, according to our experience, is undoubtedly very numerous in the Spring and Autumn, the seasons when grouse disease is most frequently observed, and practically disappears from the bird during the winter months. On the other hand it has not appeared to be more numerous in diseased than in healthy birds. *Davainea* not infrequently occurs in such enormous masses as to distend the gut.

These tape-worms have not been found associated with any constant or serious lesions. *Davainea* appears to us to be the less objectionable. *Hymenolepis*, whose seasonal prevalence more closely agrees with that of grouse disease, seems to us more likely to be harmful. The large masses in which it often exists in the narrow duodenum appear not unlikely to interfere mechanically with the free passage of food material. Both worms probably make a considerable demand for their own sustenance, even if they do not exert a more serious injurious influence.

The case against the nematode, *Trichostrongylus pergracilis*, is much clearer, for though it is seldom entirely absent from normal birds, nevertheless, definite lesions in the caecum are often associated with its presence in large numbers. It probably, however, does little harm if not too numerous. With regard to the presence of this parasite in large numbers in some of the birds caught on the moor, and supposed to be normal birds, it must be remembered that strong wild grouse are difficult to catch, and that some at least of the methods of capturing grouse alive seem calculated to catch the weakest birds rather than the stronger ones. On the other hand we have counted the strongyli in a number of "normal" and diseased birds, and have found, on the whole, a great difference between the two classes; very large numbers

being always found in the diseased birds, much larger indeed than those found in all but the exceptional members of the healthy class; and these, for reasons just stated, may perhaps be not normal at all but suffering from the early stages of grouse disease.

These nematodes, in birds picked up dead or brought to us by the keepers as suffering from grouse disease, are, so far as our experience goes, almost always associated with grave changes in the mucous membrane of the caecum; and concurrently with these changes intestinal bacteria, particularly those belonging to the *B. coli* group, find their way into the liver, or even into the other organs. We have determined by actual worm counts and cultures that *B. coli* is always absent from the liver (in birds examined immediately after death) when there are no strongyli (hand-reared birds) or only very few (not exceeding 100 in number). When more than 100 but less than 1000 are found then *B. coli* is sometimes present in and sometimes absent from the organs, but when the numbers of strongyli exceed 1000 then *B. coli* is always present in the liver, and occasionally in the other organs.

We have not been able to satisfy ourselves that the bacilli which find their way into the organs do much harm. Some harm no doubt they do, but how much we cannot say. Microscopic examination has not revealed any profound changes in these livers. The numbers in which these bacteria penetrate into the organs is difficult to estimate because, doubtless, they soon get killed in the living tissues, so that the numbers of colonies cultivated must bear only a small proportion to the total number of bacteria which have entered the fragment of tissue examined. The number of *living* bacilli in the organs of these grouse is undoubtedly small; from which it is evident that they do not multiply in the organs. Grouse disease is therefore not an *infection* with these bacteria. Is it a toxæmia caused by the poison liberated from bacteria which have been absorbed from the intestine, and which have almost immediately perished in the tissue? We know that in order to produce serious mischief in animals by a single injection of dead bacteria a considerable quantity must be employed; and it is difficult to believe, when we remember the small numbers of colonies which grew on our cultures, that relatively to this quantity the numbers of bacteria absorbed could have been very large. On the other hand we have little information concerning the influence of the constant absorption of small numbers of bacteria, but this is believed by Adami and his school to be a potent source of disease. The fact that we have repeatedly found *B. coli* in the livers of "normal"

birds badly infected with strongyli prevents us from ascribing the death of the grouse directly to these bacilli, though they probably play some part.

Conclusions.

It seems to us quite certain that the strongylus when exceptionally numerous injures the mucous membrane of the caeca, and that this injury allows of the absorption of intestinal micro-organisms. It doubtless allows also of the absorption of other substances of an irritating or poisonous nature, and probably interferes with the normal selective absorption of nourishment. If we are right in thinking that the caecal contents become partly retained, and stick to the absorbing surfaces of the ridges of the mucous membrane, we have still more reason to believe that nutrition is greatly interfered with.

"Grouse disease," as we know it, appears to us not to be a specific bacterial infection. We conceive that all the birds which are more or less severely affected by strongyli suffer injury from this cause to an extent, which is more or less proportional to the severity of the infection. Some exceptionally strong birds may stand a larger infection better than weaker birds will stand a lesser; but, on the whole, the birds with the largest numbers of strongyli suffer most. Their nutrition gets impaired owing to interference with the normal absorption of digested food, and to the abnormal absorption of soluble poisons and intestinal bacteria. Such birds become the weakest; and when food becomes scarce, as it does at the beginning of spring, especially after bad winters or on overstocked moors, or when other harmful influences prevail, it is the weakest birds which suffer most. They die of privation acting on a constitution already weakened by the consequences of strongylosis, while their stronger neighbours manage to pick up a living somehow, and tide over the period of distress.

TABLE IX, Summary of all observations.

Trichostrongylus pergracilis—In Grouse Nos. 1—41 the strongyli were not counted, and only portions of caecal contents were examined.

In Grouse Nos. 43—68 (except 45) the strongyli were counted in both separately (see p. 12).

In Grouse Nos. 69—81 (indicated * in Table) the strongyli were counted in caecum and the number found doubled.

Number	Date	Locality and History	Sex	Wt. in ozs.	Intestinal worms			Cultures from Liver
					<i>Hymenolepis</i>	<i>Davainea</i>	<i>Strongylus</i>	
1	5. v. 1908	Inverness. Caught unable to fly	♂	16	Numerous	—	Numerous	<i>B. coli</i> (12)
2	6. v. 08	„ „ „	♂	16	„	Numerous	„	<i>B. coli</i> (seve
3	„	„ „ „	♂	—	„	Few	„	<i>B. coli</i> (12)
4	„	„ „ „	♂	20	„	Numerous	„	<i>B. coli</i> (few)
5	7. v. 08	Normal hand reared bird. Frimley	—	15	0	0	—	0
6	„	Inverness. Caught unable to fly	♂	16	Numerous	Numerous	Numerous	<i>B. coli</i> (seve
11	9. v. 08	„ „ „	♂	17	„	„	„	<i>B. coli</i> „
12	„	„ „ „	♀	—	„	„	„	<i>B. coli</i> (few)
13	„	„ „ „	♀	18	„	0	„	<i>B. coli</i> (1)
14	„	„ „ „	♂	16	„	0	„	<i>B. coli</i> (1) s
15	„	„ „ „	♂	23	„	Numerous	„	<i>B. coli</i> (seve
16	2. vi. 08	Normal. Frimley ...	♂	15	0	0	Few	—
17	10. vii. 08	„ „ „	♂	14	Few	1	Eggs only	sb
18	„	„ „ „	♂	21	0	0	0	sb
19	24. vii. 08	„ „ „	♀	23	0	0	0	0
20	28. vii. 08	„ „ „	—	15	0	0	Few	—
21	5. viii. 08	„ „ „	♀	15	0	0	0	0
22	10. viii. 08	„ „ „	♀	—	0	2	Numerous	<i>B. coli</i> (1)
23	15. viii. 08	Inverness. Apparently healthy	♂	13	Numerous	Numerous	0	<i>B. coli</i> (4)
25	18. viii. 08	Normal. Frimley ...	♂	15	0	0	0	0
26	„	„ „ „	♀	13	0	1	0	<i>S. lutea</i> (1)
28	—	Inverness ...	—	—	0	0	Numerous	<i>B. coli</i> (few)
29	27. viii. 08	„ Caught unable to fly ...	—	15	0	0	„	<i>B. coli</i> (1)
30	26. viii. 08	„ Apparently healthy ...	♀	16	Few	Numerous	Few	0
31	27. viii. 08	„ „ „	♀	20	Numerous	Few	Numerous	0
32	28. viii. 08	„ Healthy, caught on moor	♀	16	Moderate	Moderate	Moderate	<i>B. coli</i> (2)
33	„	„ „ „	♂	15	Numerous	Numerous	Few	<i>B. coli</i> (few)
34	29. viii. 08	„ „ „	♂	18	Few	Moderate	„	0
35	„	Nairn. Caught on moor. Ill ...	♀	17	Numerous	Numerous	Numerous	<i>B. coli</i> (seve
36	3. ix. 08	Normal bird. Examined 4 days after death	♀	—	0	0	0	—
37	14. ix. 08	Normal bird. Found dead. Frimley	♀	11	0	0	0	—
38	23. x. 08	Normal bird. Died of pericarditis	♂	20	0	Numerous	Numerous	st.c (1)
39	24. x. 08	Lancashire. Caught unable to fly	♂	18	Few	„	6230	—

TABLE (continued).

B. enter. = Bacillus of the *B. enteritidis* group.*c* = coccus.*m* = mould.*sb* = spore-bearing bacillus.*sx* = streptothrix.*b* = bacillus.*d* = diphtheroid bacillus.*s* = sarcina.*st.c* = streptococcus.

The numbers or words in brackets indicate the number of colonies found.

Cultures from organs							
gs	Kidneys		Spleen	Pancreas	Bile	Blood	Remarks
	Left	Right					
0	—	—	—	—	—	—	Examined 2 hours after death. Moderate reddening of caecal villi.
0	—	—	—	—	—	—	Caecal mucous membrane much congested.
0	—	—	—	—	—	—	Caecal mucous membrane little congested.
<i>stc</i> (2)	—	—	—	—	—	—	Caecal mucous membrane not congested.
0	—	—	—	—	—	—	" " " "
0	—	—	—	—	—	—	—
<i>b</i>	—	—	—	—	—	—	Caecal mucous membrane much congested.
0	—	—	—	—	—	—	Caecal mucous membrane slightly congested.
—	—	—	—	—	—	—	Caecal mucous membrane not congested.
0	—	—	—	—	—	—	" " " "
<i>B. coli</i>	—	—	—	—	—	—	Caecal mucous membrane extremely congested.
—	—	—	—	—	—	—	Ditto, not congested. ? Gut wounded while making cultures.
<i>sx</i>	<i>sb</i>	<i>sb</i>	<i>sb</i>	—	0	0	Caecal mucous membrane not congested.
<i>sb</i>			<i>m</i>				
<i>sb</i>	<i>sx</i>	0	0	—	<i>sb</i>	0	" " " "
0	0	0	<i>sb</i> (1)	—	0	0	—
0	0	0	—	—	—	—	Gut wounded when making cultures.
0	0	0	0	—	0	—	—
<i>m</i> (1)	<i>B. coli</i> (2)	0	0	—	0	—	—
<i>c</i> (1)							
<i>m</i> (1)	<i>sb</i> (1)	0	<i>B. coli</i> (1)	—	0	—	Numerous portions of <i>Davainea</i> in caeca.
<i>m</i>	0	<i>c</i> (1)	0	—	0	—	—
<i>sb</i>							
<i>m</i> (1)	0	0	0	—	0	—	—
<i>sx</i> (1)							
<i>sb</i>	0	0	0	—	—	<i>c</i> (1)	Shot in wing. Brought in alive.
<i>sx</i> (1)	0	0	0	—	—	—	—
<i>m</i> (1)	<i>sb</i> (1)	0	0	—	—	—	—
<i>m</i> (1)	0	0	<i>B. coli</i> (1)	—	—	—	—
<i>sb</i>							
<i>m</i> (1)	<i>sb</i> (1)	0	0	—	—	—	—
<i>sb</i> (1)	0	0	<i>s</i> (1)	—	—	—	—
0	<i>sb</i> (1)	0	0	—	—	—	—
<i>b</i> (1)	0	0	0	—	—	0	Caecal mucous membrane somewhat reddened.
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
0	0	0	—	—	—	—	—
—	—	—	—	—	—	—	Arrived dead.

TABLE (continued).

Number	Date	Locality and History		Sex	Wt. in ozs.	Intestinal worms			Cultures fr Live
						<i>Hymenolepis</i>	<i>Davainea</i>	<i>Strongylus</i>	
40	28. x. 1908	Normal. Died of pneumonia.	Frimley	♂	20	0	0	Few	0
41	„	Lancashire. Caught unable to fly		♂	20	0	Moderate	Numerous	—
43	8. xii. 08	Cumberland. Normal		♂	17	0	1	45	0
44	„	„ „ „		♀	19	0	0	871	<i>B. coli</i> (4)
45	„	Nairn. Picked up dead		♂	16	0	Moderate	Numerous	—
46	9. xii. 08	Cumberland. Normal		♂	16	0	0	259	<i>B. coli</i> (1)
47	„	„ „ „		—	18	0	0	344	<i>B. enter.</i>
48	„	„ „ „		♂	—	0	0	32	s (2)
49	„	„ „ „		♀	13	0	Several	330	c (1)
50	„	„ „ „		♀	19	0	0	290	<i>B. coli</i> (3)
51	„	„ „ „		♀	13	0	0	540	<i>B. coli</i> (1)
52	21. xii. 08	Lancashire. Normal. Hand reared		♂	15	0	0	0	0
53	—	Montgomeryshire. Picked up dead		♂	16	0	0	2506	—
54	3. ii. 09	Cumberland. Normal		♀	16	0	6	1645	<i>B. coli</i> (1)
55	„	„ „ „		♂	17	0	7	415	0
56	5. ii. 09	„ „ „		♂	16	0	5	1868	<i>B. coli</i> (1)
57	„	„ „ „		♀	17	0	11	599	<i>B. coli</i> (1)
58	„	„ „ „		♀	17	0	0	113	0
59	6. ii. 09	„ „ „		♀	12	0	0	235	<i>B. coli</i> (1)
60	17. iii. 09	Yorkshire. Normal. Caught on moor		♀	21	Few	Numerous	5995	<i>B. coli</i> (1)
61	„	„ „ „		—	21	Numerous	Numerous	9562	<i>B. coli</i> (1)
62	19. iii. 09	„ „ „		♀	21	0	0	730	<i>B. coli</i> (1)
63	20. iii. 09	Selkirk. Normal. Caught on moor		♀	18	Numerous	0	533	c (1)
64	„	„ „ „		♀	20	„	Numerous	571	0
65	„	Cumberland. Shot		♀	18	„	2	183	—
66	„	Selkirk. Normal. Caught on moor		♀	19	„	1	945	0
67	„	„ „ „		♀	18	„	Moderate	833	0
68	22. iv. 09	Caithness. „ „		♀	16	0	0	877	—
69	27. iv. 09	Inverness. „ „		♂	23	Numerous	Numerous	730*	—
70	„	„ „ „		—	23	„	„	2524*	—
71	10. v. 09	„ Caught, unable to fly		♀	16	„	0	7058*	—
72	„	„ Picked up dead.		♂	17	„	Numerous	10266*	—
73	7. v. 09	Nairn. „ „		♀	17	„	Fragments	7484*	—
74	21. v. 09	Yorkshire. „ „		♀	17	0	Moderate	3114*	—
74 (a)	„	„ „ „		—	—	Numerous	0	3406*	—
75	19. v. 09	„ „ „		♂	—	„	Numerous	4352*	—
76	„	Lancashire. „ „		—	—	0	„	18332*	—
77	„	Selkirk. „ „		♂	21	Numerous	0	8800*	—
78	24. v. 09	„ „ „		♀	16	„	Few	3340*	—
79	3. vi. 09	Inverness. „ „		♀	17	Few	0	2556*	—
80	„	„ „ „		♂	19	0	0	3840*	—
81	17. xii. 09	Normal. Frimley		♀	15	0	0	0	0

TABLE (continued).

Cultures from organs							
gs	Kidneys		Spleen	Pancreas	Bile	Blood	Remarks
	Left	Right					
<i>m</i> (1)	—	—	0	—	0	0	—
—	—	—	—	—	—	—	Arrived dead.
<i>st.c</i> (1)	0	0	—	—	—	—	—
<i>m</i> (1)							
<i>sx</i> (1)							
<i>m</i> (1)	0	0	0	0	—	0	—
—	—	—	—	—	—	—	—
<i>sx</i>	0	0	0	—	—	0	—
<i>m</i> (1)	0	<i>sb</i> (1)	<i>B. enter.</i>	—	—	—	Patches of dry faecal matter adhering to caecal wall.
<i>c</i> (1)							
<i>sx</i>	0	<i>sb</i>	—	0	—	—	—
<i>m</i>	<i>c</i> (1)	0	0	0	—	—	—
<i>m</i>	0	0	<i>B. coli</i> (6)	0	—	—	—
<i>sx</i>							
<i>sx</i>	<i>B. enter.</i>	0	<i>B. enter.</i>	<i>B. enter.</i>	—	—	Bird in very poor condition.
<i>m</i>		<i>B. enter.</i>					
<i>B. enter.</i>							
<i>m</i>	0	0	0	0	—	—	32 specimens of <i>Trichosoma longicolle</i> in duodenum. 32 specimens of <i>Heterakis papillosa</i> in caeca.
<i>c</i>							
—	—	—	—	—	—	—	2 specimens of <i>Trichosoma longicolle</i> in duodenum.
<i>m</i>	0	0	<i>B. coli</i> (3)	—
<i>sx</i>	0	0	—	<i>s</i> (1)	—	—	—
<i>m</i>							
<i>c</i>	—	0	—	—	—	—	—
<i>m</i>	0	—	0	0	—	—	—
<i>sx</i>	—	<i>s</i> (1)	0	—	—	—	—
<i>m</i>							
<i>sx</i>	0	0	0	—	—	—	1 specimen of <i>Heterakis papillosa</i> in each caecum.
<i>m</i> (1)	0	—	—	—	—	0	„ „ „ „
0	0	0	0	<i>s</i> (1)	—	0	—
<i>sx</i>	0	0	—	0	—	0	—
<i>m</i>							
<i>sx</i>	<i>c</i> (1)	0	0	0	—	0	—
0	0	0	0	0	—	0	—
—	—	—	—	—	—	—	—
<i>m</i> (1)	0	0	—	0	—	0	—
0	0	0	0	0	—	0	—
—	—	—	—	—	—	—	Arrived dead.
—	—	—	—	—	—	—	„
—	—	—	—	—	—	—	„
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
<i>m</i>	0	0	0	—	0	0	—

EXPLANATION OF PLATES I—V.

- Fig. 1. ($\frac{1}{2}$ nat. size) showing the glass frame in which the crushing of the tissues was done. On the ledge are three pairs of ground glass plates. One of the plates of the pair on the left is being held up in the manner in which this was done preparatory to placing a piece of tissue between the plates.
- Fig. 2. ($\frac{1}{3}$ nat. size) showing the alimentary canal of a normal grouse (No. 81) from the gizzard to the anus. Gizzard (a), duodenum, enclosing the pancreas (b), intestine (c), caeca (d, d) and rectum (e).
- Fig. 3. ($\frac{1}{2}$ nat. size) showing small test tubes arranged in pairs containing the strongyli collected from the two caeca of eleven grouse. The serial number of the grouse is written on the top, and the number of strongyli present in each tube in smaller figures below.
- Fig. 4. ($\frac{1}{2}$ nat. size) showing small test tubes containing the strongyli collected from both caeca of 18 grouse. No strongyli were found in the caeca of grouse No. 52, and in this case the test tube contains 32 specimens of *Heterakis papillosa*. The serial number of the grouse is printed above each tube.
- Fig. 5. ($\times 9$) showing a transverse section of the caecum of a normal grouse (No. 81). The ridges (a) and villi (b) are well shown, and three collections of lymphoid tissue (c) are also seen.
- Fig. 6. ($\times 25$) showing a portion of the same section more highly magnified.
- Fig. 7. ($\times 25$) showing a portion of a section of the caecum of an apparently healthy wild grouse (No. 69).
- Fig. 8. ($\times 9$) showing a transverse section of the caecum of a diseased grouse (No. 2).
- Fig. 9. ($\times 9$) showing a transverse section of the caecum of a diseased grouse (No. 15) very badly infected with strongyli.
- Fig. 10. ($\times 25$) showing a portion of Fig. 9 more highly magnified. Large numbers of worms are seen in transverse section as black dots.
- Fig. 11. ($\times 5$) showing the internal surface of the caecum of a normal grouse (No. 81) after gentle washing. Several ridges are seen, some of which die away near the centre of the specimen. The whole surface is covered with small villi.
- Fig. 12. ($\times 5$) showing the internal surface of the caecum of an apparently healthy wild grouse (No. 69) after gentle washing. The ridges are greatly developed, and the villi larger and more prominent than in the preceding figure. A few worms can be seen.
- Fig. 13. ($\times 5$) showing the internal surface of the caecum of a diseased grouse (No. 73) after gentle washing. The ridges are very broad, and the villi in some places hypertrophied (a). In one situation the villi are so matted together that they are almost indistinguishable (b). At this spot a mass of dry material adhered to the ridge.
- Fig. 14. ($\times 5$) showing the internal surface of the caecum of a diseased grouse (No. 12) after gentle washing. The ridges are very prominent, but the villi are matted together to such a degree with cementing material that they are almost indistinguishable. Some of the ridges are united with the same material (a, b, c).
- Fig. 15. ($\times 100$) showing two specimens of *T. pergracilis* (a, b) in section in the epithelium covering a villus.
- Fig. 16. ($\times 100$) showing a specimen of *T. pergracilis* (a) in section surrounded by a ring of fibrous tissue (b).
- Fig. 17. ($\times 5$) showing large numbers of *T. pergracilis* on the internal surface of the caecum of a diseased grouse (No. 13).
- Fig. 18. ($\times 100$) showing a specimen of *T. pergracilis* (in section) between two villi. The epithelium has been lost and fibrous tissue (a) has been formed within one of the villi in the neighbourhood of the worm.
- Fig. 19. ($\frac{2}{3}$ nat. size) showing two tubes containing the specimens of *Davainea urogalli* (on the left) and of *Hymenolepis microps* (on the right) obtained from grouse No. 11.
- Fig. 20. (nat. size) showing a tangled mass of *Davainea urogalli* (partially opened out) from the intestine of a grouse.



Fig. 1.



Fig. 2.

65 59 55 63 57 64
89—94 107—108 201—214 252—281 268—331 268—303

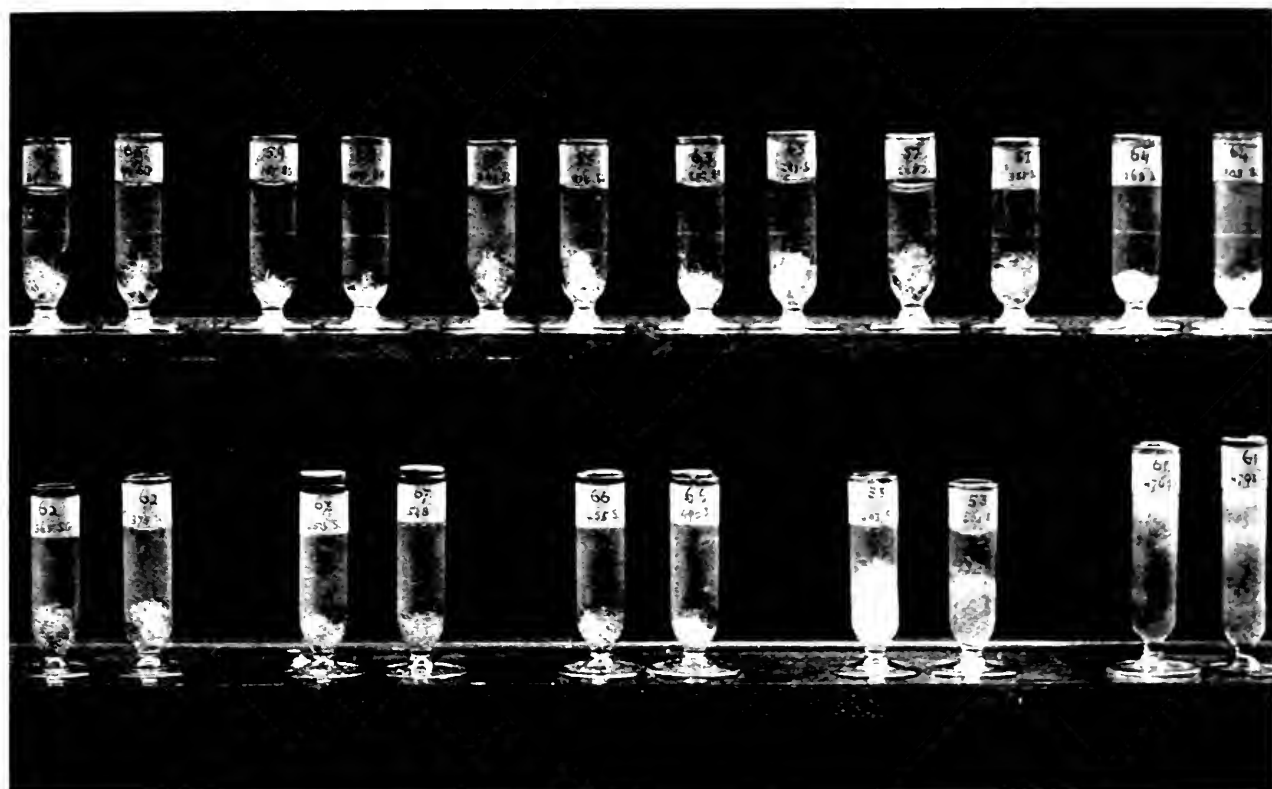
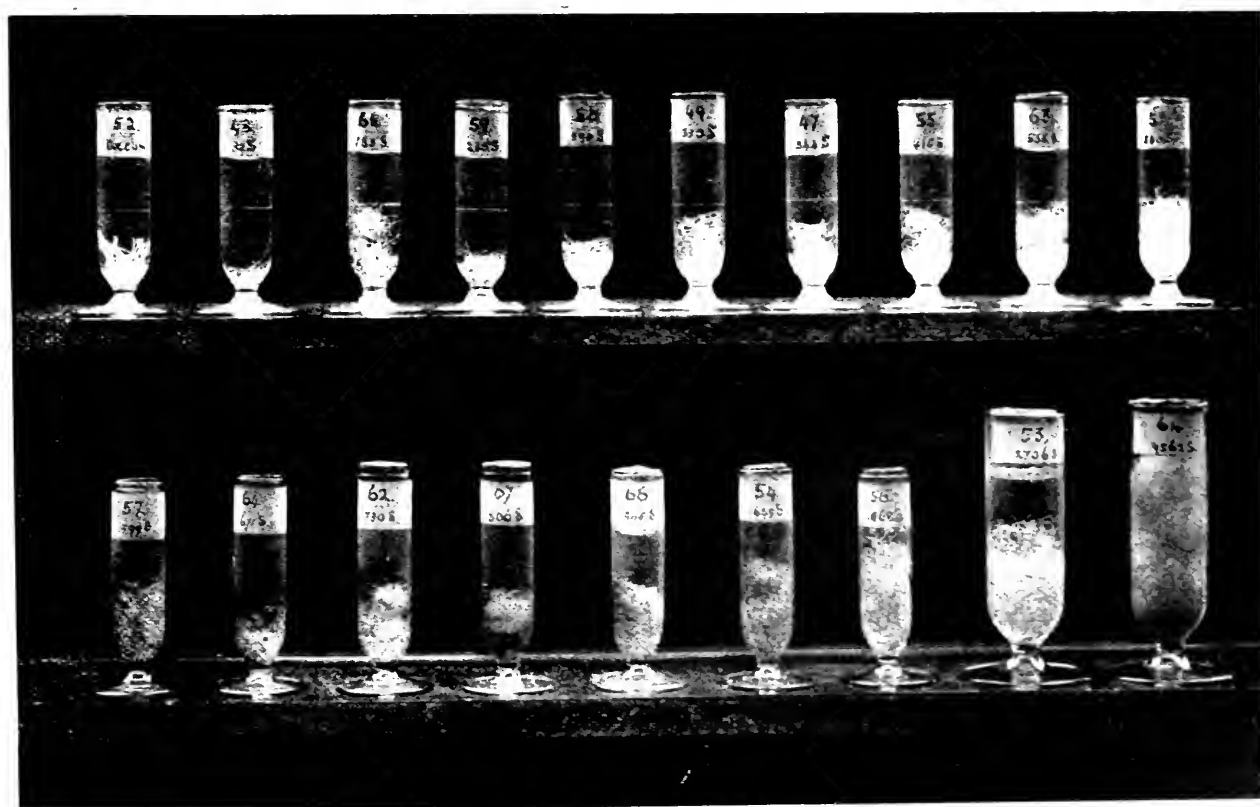


Fig. 3.

52 48 65 59 50 49 47 55 63 51



57 64 62 67 66 54 56 53 61

Fig. 4.



c a b

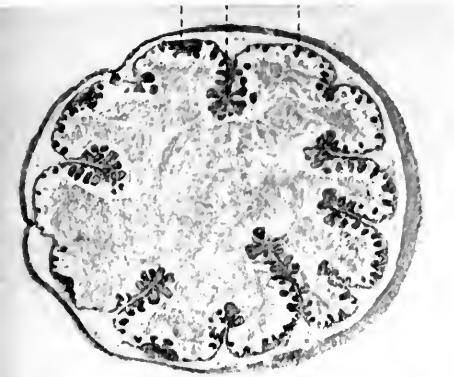


Fig. 5.

c a b

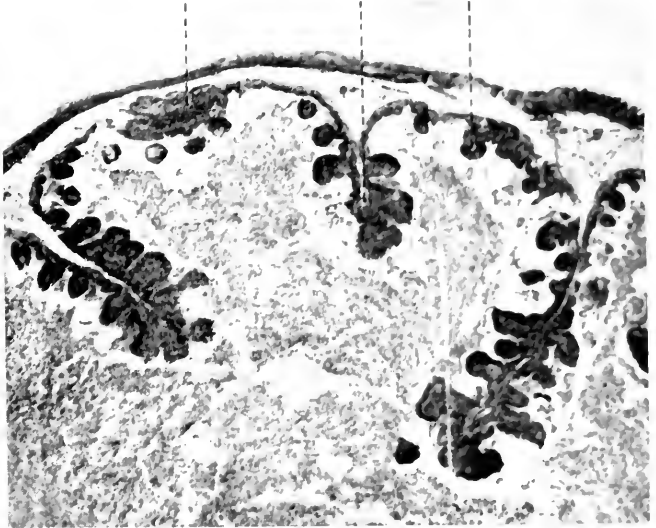


Fig. 6.

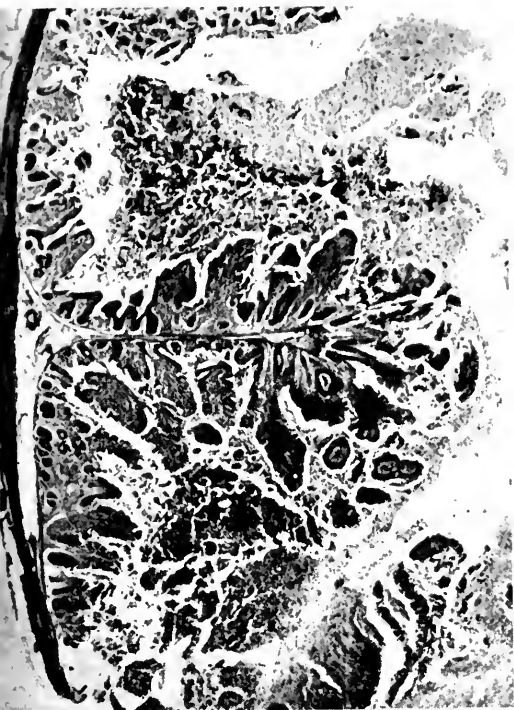


Fig. 7.



Fig. 8.



Fig. 9.



Fig. 10.



Fig. 11.



Fig. 12.

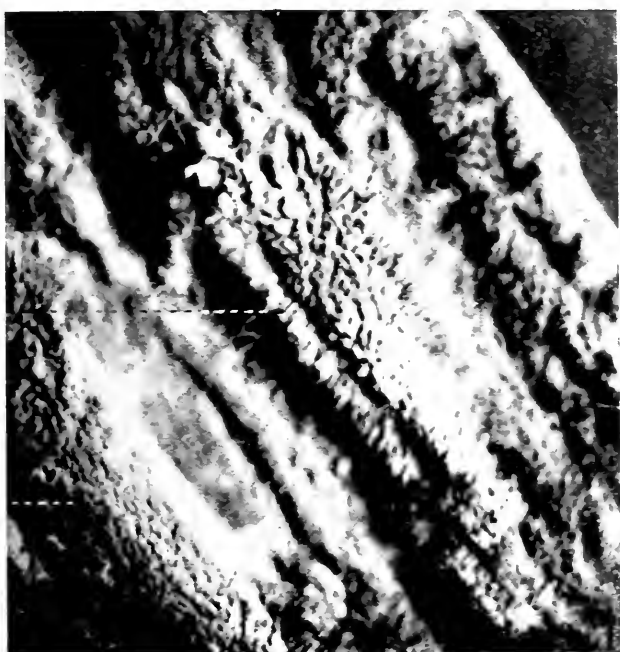


Fig. 13.

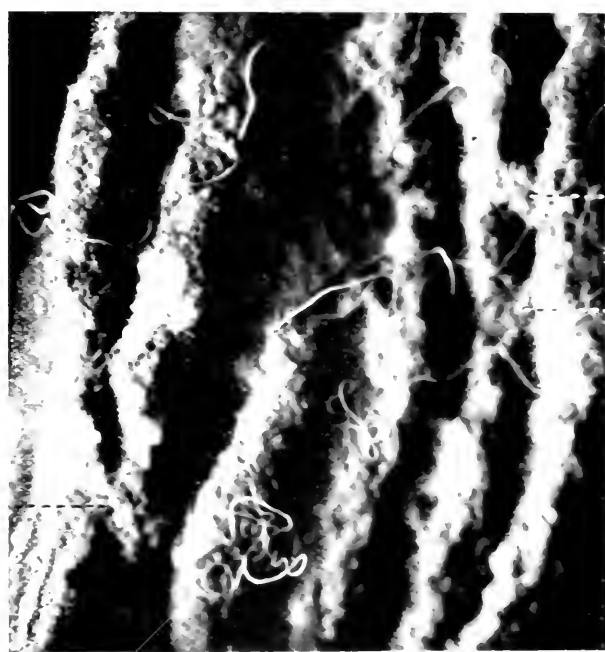


Fig. 14.



Fig. 15.



Fig. 16.



Fig. 17.



Fig. 18.

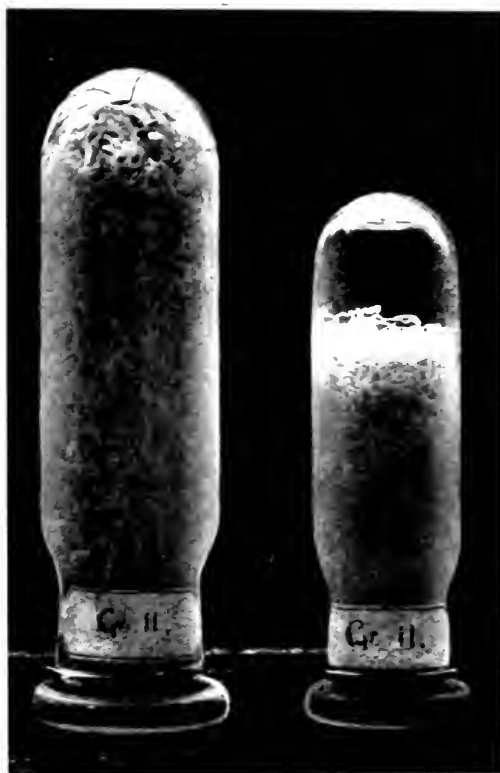


Fig. 19.



Fig. 20

AN INVESTIGATION INTO THE ACID-FAST BACTERIA FOUND IN HUMAN FAECES WITH SPECIAL REFER- ENCE TO THEIR PRESENCE IN CASES OF TUBERCU- LOSIS.

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UP TO the year 1906 tubercle bacilli were considered to occur in the faeces only in pulmonary or abdominal tuberculosis, due to ingestion of the infected sputum or to ulceration of the intestinal tract.

Wood (1905) for instance writes that tubercle bacilli are to be found in the faeces of persons suffering from pulmonary tuberculosis because in the majority of cases the bacilli are swallowed in small masses of sputum. Sahli (1906) states that they may occur in the faeces in intestinal tuberculosis and that "the stools may contain the bacilli even though there is no intestinal tuberculosis (if the patients swallowed their sputum)." He gives the warning that "the tubercle bacillus must be carefully distinguished from the smegma bacillus which is said to occur at the anal orifice, and might have become mixed with faeces." Lichtheim (1883) and Shaw (1897) are in agreement with these opinions, Boston¹ and Simon¹ favour the idea that the appearance of the bacilli in the faeces is strong evidence of intestinal ulceration. The former believes that their presence "points conclusively to the existence of tuberculous ulceration of the intestines"; the latter agrees with this statement if clinical symptoms point to the same conclusion.

Emerson (1906) showed that numerous bacilli in the faeces did not necessarily imply ulceration of the intestine. He demonstrated the

¹ Cited by Rosenberger (1907).

presence of acid-fast bacilli in 57 cases of pulmonary and abdominal tuberculosis. The post-mortem examinations of 33 of these revealed no ulceration of the intestine. Bodo (1892) is quoted as having examined the faeces of nine persons suffering from pulmonary phthisis, finding tubercle bacilli in three.

These observations show (1) that acid-fast bacilli may be present in the faeces in cases of pulmonary and intestinal tuberculosis, and (2) that their presence even in relatively large numbers does not necessarily imply ulceration of the intestine. They do not show that these acid-fast bacilli are tubercle bacilli.

Rosenberger (1907—1909) has made further progress in these investigations. He showed, firstly, that acid-fast bacilli could be demonstrated in the blood of tubercular patients, especially in cases of miliary tuberculosis. This demonstration of acid-fast bacilli in the blood-stream by staining methods has not been confirmed by other observers. It is certain however that they must be of frequent occurrence if the organisms are to be found in the faeces in any numbers. In this connection Rosenberger's observations upon cases of acute miliary tuberculosis are interesting. He found acid-fast bacilli in the faeces in every case. It is only by reference to the circulating blood that it is possible to explain the presence of these bacilli in the alimentary canal when the tubercular focus is in a distant region of the body. If the affected area be situated in the hip-joint or in the dorsal vertebrae, as was the condition in two of Rosenberger's cases, the blood-stream must eventually if not primarily, have been the path by which the specific bacillus reached the intestine. It becomes apparent, if these things be true, that not only must the old ideas regarding the appearance of the bacilli in the excreta be abandoned but also the conception of the bacilli in the blood must be changed and it be admitted that they occur far more frequently than was hitherto deemed possible.

Rosenberger showed secondly that acid-fast bacilli could be found in the faeces of patients suffering from tuberculosis other than that of the lungs or intestine.

He examined in all 672 cases of various forms of the disease and found an acid-fast bacillus in 120 of them. Of these 120 positive cases, 60 had been previously diagnosed as tubercular, the remaining 60 were suspicious cases.

Rosenberger never produced tuberculosis in animals with the acid-fast bacillus which he observed in the faeces. He therefore never proved that they were tubercle bacilli.

So far as the present writer can discover human faeces have never been examined with the view of establishing the identity of any acid-fast bacilli they might contain, except in three cases of typhoid to which reference will be made later.

Animal faeces have been examined twice with this object, by Schroeder and Cotton (1907) and by Griffith (1909). Naturally tuberculous cows were examined, and in a large percentage the faecal matter proved capable of producing infection when injected into guinea-pigs and rabbits, or fed to swine.

The Objects of the Research.

(1) Primarily to prove or disprove the identity of acid-fast bacilli in faeces with the tubercle bacilli. As this could only be accomplished by animal inoculation preliminary investigations were necessary to discover:

(a) What quantity of human faeces could be inoculated into guinea-pigs without producing fatal septicaemia.

(b) Under what conditions of the faecal contents acid-fast bacilli were most numerous.

After ascertaining these it remained to show that:

(2) Acid-fast bacilli are not found in the faeces under normal conditions or in diseases other than tuberculosis.

(3) The acid-fast bacillus present in the faeces of tuberculous persons were tubercle bacilli.

Observations were also made regarding:

(4) The proportion of cases of tuberculosis in which acid-fast bacilli could be demonstrated by staining methods in the faeces.

PRELIMINARY INVESTIGATIONS.

1. *Dilution of the faeces.*

The amount of the injection proved a great difficulty at first. It was considered necessary to find the largest amount of diluted faeces which the animal could survive. It was feared that with too high a dilution, the bacilli which were never present in any numbers would be lost. After several unfortunate results it was found that 0.02 gramme was the largest amount of human faeces that could with safety be injected

subcutaneously into a guinea-pig of average weight. The animal survived occasionally after the injection of 0·04 gramme. Death ensued occasionally after the injection of 0·01 gramme. The variations in the virulence of the faecal organisms and the resistance of the animals evidently swung within wide limits. It was deemed advisable to inoculate at least two animals from each sample. One received 0·02 gramme, the other 0·01 gramme. The routine employed was as follows:

A gramme of faeces was carefully weighed out on a watch glass, rubbed up in a mortar with normal saline and further diluted until the final volume reached 99 c.c. This gave a dilution of one in a hundred. (All apparatus used had been previously sterilised and the operations were carried out as rapidly as was consistent with accuracy.) Of this dilution 1 c.c. was injected into one animal, 2 c.c. into a second.

2. *The conditions under which the faeces contained the greatest number of acid-fast bacilli.*

It was noted that the demonstration of acid-fast bacilli was difficult or impossible in such stools as were hard and constipated and a reference to Rosenberger's paper made it plain that he had experienced a similar difficulty. He states that the bacillus was found "in solid stools 28 times; in semi-solid stools 40 times and in fluid stools 52 times." No mention is made of the use of purgatives so it must be concluded that none were employed. It is evident that there are circumstances connected with the formation of constipated stools which may lead to one of two conditions.

1. The bacilli are present but may not be demonstrated.
2. The bacilli are not present.

In discussing the probabilities of the first supposition it will be remembered that the *viable* bacteria of a loose motion and those of a constipated stool differ greatly in number. Whereas in the former 10—15 % of the total organisms may be living, in the latter only 2 % of the bacteria seen in a stained smear may be induced to form colonies in the most suitable medium known.

Are then the forces at work in a constipated stool powerful enough to kill the tubercle bacillus or can they destroy its staining reaction?

To elucidate this question an attempt was made to produce constipation *in vitro*, if such a term may be used.

Stools were taken from advanced cases of pulmonary tuberculosis. These were examined microscopically and shown to contain the organisms in numbers. They were allowed to remain in the bottles or jars in which they had been received, for varying periods under varying conditions of temperature. At the end of the time they were softened with normal saline, gently ground up in a mortar and fresh smears examined.

Table I shows the results of these experiments.

TABLE I.

Case	Faeces before	Period days	Conditions	Faeces after
A	++	7	Ice chest	+
B	++	10	„	++
C	+++	15	Incubator 37°	++
D	++	15	Ice chest	+
E	++	19	„	+
F	++	20	Room-temp.	+
G	+	20	„	+
H	+++	20	Incubator 22°	++
I	++	20	Ice chest	+

+++ = very numerous. ++ = numerous. + = present.

Acid-fast bacilli were demonstrated in every specimen after it had become hard and dry.

There is a noticeable diminution in the numbers that could be demonstrated.

Are the bacilli present in constipated stools?

Constipation is usually accompanied and frequently caused by lack of bile. Lack of bile is one of many causes of constipation but a recollection of Calmette's and Guérin's work (1909) on the liver drew attention in this direction. The conditions which govern the excretion of the typhoid bacillus are intimately connected with the flow of bile and the excretion is intermittent.

An intermittency in the excretion of the tubercle bacillus, if present, would suggest, among other things, that the bile had some connection with its excretion.

But first this intermittency had to be looked for. The consecutive motions of two men, both advanced cases, were examined. During the period over which these investigations extended no purgative was administered.

Acid-fast Bacteria

Case *A* had both lungs affected. Motions were easy and normally regular and he rarely required a purgative.

Case *B* suffered from constipation and frequently asked for a purgative. The results are given in the following Tables (II and III).

TABLE II. *Case A.*

Stool	Passed	Consistency	Faeces
1	a.m.	semi-solid	+
2	„	„	+
3	„	solid	—
4	„	semi-solid	+
5	„	„	+
6	„	„	—
7	„	„	+
Total	7		Total 5

TABLE III. *Case B.*

1	a.m.	solid	—
2	p.m.	„	—
3	a.m.	semi-solid	—
4	„	„	+
5 & 6	a.m. & p.m.	fluid (diarrhoea)	+ a.m. & p.m.
7	a.m.	semi-solid	+
8	„	solid	—
Total	8		Total 4

Case *A* excreted the bacillus more regularly than *B*, in whose faeces the bacillus only appeared with the onset of diarrhoea.

Both *A* and *B* were given half a grain of calomel at night and a seidlitz powder in the morning. The results are given below.

Case A. Calomel and Seidlitz.

Stool	Description of stool	Faeces
1	semi-fluid	+
2	„	++
3	„	++
4	„	+
5	„	+
6	„	+
7	„	+

Case B. (Same treatment.)

Stool	Description of stool	Passed
1	semi-fluid	+ +
2	„	+
3	„	+
4	„	+
5	„	+ +
6	„	+
7	„	+

This purgation, which might seem severe, produced a considerable improvement in the condition of the patients. Their evening temperature was lowered and they confessed to feeling much better in every way. The faeces of “B” were now sent for examination after administration of Mist. Alba.

Stool	Description of stool	Purgative	Faeces
1	soft formed	Mist. Alba	+
2	„	„	+
3	semi-solid	„	—
4	soft formed	„	+
5	„	„	—
6	„	„	+

This was not so effective as the calomel.

Are acid-fast bacilli normally present in faeces?

It has been suggested, apparently without any authority that could be quoted, that acid-fast bacilli occur in normal faeces. Only two references have been found after an extended search in the literature to anyone who has demonstrated acid-fast bacilli in faeces other than those from a tuberculous individual. Sahli has been quoted before in this paper as mentioning that the smegma bacillus “is said to occur at the anal orifice” but even he affords no confirmation that they do actually occur. Jousset (1903) found acid-fast bacilli in the stools of two typhoid patients. They were easily distinguishable from tubercle bacilli by their shape, arrangement, growth on potatoe, and by failure to produce lesions in guinea-pigs. Mironescu (1901) also found an acid-fast bacillus in a case of suspected typhoid. It was ultimately proved to be the acid-fast bacillus found in butter.

It has been the author's custom for over two years to stain smears of every specimen of faeces, which came for examination, in at least three ways: (1) by gram, counterstaining lightly with weak carbol-fuchsin, (2) with Leishmann's stain, and (3) with Ziehl-Neelson.

Hence at hand there are numerous pathological conditions of the intestinal tract the excreta of which have supplied samples, all of which have been examined for acid-fast bacilli. In all there are records of 129 faeces so examined. Of these 69 have been examined especially for this research and all have undergone a careful search.

The 129 include 18 healthy normal faeces, 9 epileptic faeces, 28 suspected tubercle, and smaller numbers of such cases as typhoid, acne, mucous colitis, acute osteo-myelitis, diabetes, cholelithiasis, and one vegetarian stool.

It may be mentioned here that there occurs numerously in some faeces and not at all in others, oval bodies which are acid-fast. These are homogeneous in structure and in size about the length of *Bacillus aerogenes capsulatus*. The oval is usually perfect in shape, but occasionally it tends to become pear-shaped. In the adult they occurred most numerously in the faeces of a vegetarian and in a case of acne. They are found however in greatest numbers in the stools of children. In an investigation made last year into the meconial stool, these bodies were discovered in nearly every specimen examined.

No conclusion has been reached as to their nature. Herter (1908) is the only author who has described them and he is unable to pronounce upon their identity. He thought at first they were yeasts, but being unable to procure a culture in any media, finally suggested that they were bodies ingested with the food.

The identity of acid-fast bacilli in the faeces.

The faeces of 24 persons suffering from advanced pulmonary phthisis were inoculated into guinea-pigs in the amounts determined by the preliminary investigation.

The faeces of two cases of lupus were also inoculated, in neither of these were acid-fast bacilli seen under the microscope and none of the animals inoculated showed signs of tuberculosis. Twenty-three of the twenty-four samples from advanced phthisis produced general tuberculosis in guinea-pigs and in all of these acid-fast bacilli were seen under the microscope. The twenty-fourth sample contained no acid-fast bacilli by microscopical examination, and no lesions were produced in

the inoculated animals. It is interesting to note that a sample from this last case procured a week later by the administration of a purgative revealed acid-fast bacilli.

The proportion of cases of tuberculosis in which acid-fast bacilli could be demonstrated by staining methods in the faeces.

Various techniques have been suggested for the examination of the faeces for this purpose. Hamburger, quoted by Sahli, recommended the method of Strassburger for solid stools. This consists of a fractional sedimentation in the centrifuge by which the larger particles of the faeces are first thrown down from normal saline and a final sediment procured by centrifugalisation in alcohol.

Page¹ and Park (1905) also consider this method the most suitable. Rosenberger made no use of the centrifuge in his work.

Technique.

The examination of constipated stools was soon abandoned for reasons already stated.

A sterile glass rod was employed to remove a small mass or drop from soft or liquid stools on to a glass slide. A second slide was used to smear out this mass and a film was made in a similar fashion to that by which a blood-film is produced.

This was allowed to dry in the air and fixed by heat in the Bunsen flame. It remained in steaming carbol-fuchsin for five minutes, was washed and treated with 25 % sulphuric acid until only a faint pink appeared on re-washing. It was then lightly counterstained with Loeffler's methylene blue.

Early pulmonary cases (14).

These cases all exhibited very early symptoms such as haemoptysis only, winter coughs, loss of flesh and a few with signs at one apex. Fourteen suspicious cases were examined, of these twelve were ultimately diagnosed as tubercle and nine were passing acid-fast bacilli in their faeces.

¹ Quoted by Emerson.

Bones and joints (9).

These included one case of Pott's disease and one case of psoas abscess.

Nine altogether were examined, seven were finally diagnosed tubercle, two presented acid-fast bacilli in the stool.

Lupus (6).

Six cases were examined, kindly sent to me by Dr F. P. Wilson. No acid-fast bacilli were found.

Tubercular meningitis (5).

Five cases examined, four ultimately proved to be tubercular, and an acid-fast bacillus found in the faeces of all four.

Miliary tubercle (4).

Four genuine cases all exhibited the bacillus in their motions.

Glandular enlargement (6).

Six cases examined, all tubercular. The bacillus was found on the two occasions in which the glands were very caseous.

Chronic pleurisy (6).

Six cases were examined. Four of these proved to be tubercular. Acid-fast bacilli were found in two. The non-tubercular cases consisted of one case of sarcoma of the lung and a case of chronic pneumococcal pleurisy.

Cases of pulmonary phthisis with small area of lung affected (7).

Seven examined. Acid-fast bacilli were found in the sputum in all seven, in the faeces six times.

Table summarising cases examined.

Type of lesion	Number examined	Positive examination	No. of cases of T.B.
Early pulmonary cases	14	9	12
Medium " "	7	6	7
Advanced " "	24	23	24
Bones and joints	9	2	7
Lupus	6	0	6
Meningitis	5	4	4
Miliary	4	4	4
Glandular enlargement	6	2	6
Pleurisy	6	2	4
Total	81	52	74

CONCLUSIONS.

The faeces of 23 cases of pulmonary tuberculosis, in which acid-fast bacilli had been demonstrated by the microscope, have been proved capable of infecting guinea-pigs with tubercle.

One hundred and twenty-nine samples of faeces from non-tubercular individuals have been examined microscopically and no acid-fast bacilli demonstrated.

The inference follows that all acid-fast bacilli in the faeces are tubercle bacilli.

Acid-fast oval bodies were found frequently but their nature is unknown.

The tubercle bacillus was discovered 52 times in the faeces of 74 cases, certainly tubercular.

There is an intermittency in the appearance of tubercle bacilli in the faeces, which may be connected with the flow of bile. This explanation is supported by the action of the cholagogue, calomel.

It is a pleasant duty to acknowledge the kind assistance and advice received throughout this investigation from Dr Stenhouse Williams, and to thank him for permission to work in his Laboratory.

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A CONTRIBUTION TO THE ETIOLOGY OF BERI-BERI.

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Plate VI.

No hypothesis hitherto advanced with regard to the origin of Beri-Beri, has up till now found sufficient experimental confirmation to warrant its general acceptance. This applies especially to the conception of the disease as due to the presence of bacteria or parasites in the blood, further to the infection theory as well as to the miasmatic theory¹.

One fact, however, stands out clearly and is now generally admitted by modern investigators, namely that there is some connection between Beri-Beri and rice. In all those eastern countries in which the disease is endemic, rice forms the staple food and it may be safely said that, in Japan at least, every sufferer from Beri-Beri is a rice eater and that conversely European residents who eat little or no rice do not get Beri-Beri.

In considering this subject the treatment which the rice has undergone previous to its consumption as food, plays an important rôle. The rice consumed in Japan is mainly so-called "white rice," *i.e.* rice freed from pericarp and husks, and finally "polished." As we were mainly concerned with the investigation of this kind of rice, the other qualities ("red" rice, "cured" rice, etc.) may be left out of consideration here, although their importance with regard to the origin of Beri-Beri in other eastern countries is obvious.

¹ No attempt has been made to deal in this short communication with the extensive literature of the subject. Very full references to it will be found in W. L. Braddon's monograph, *The Cause and Prevention of Beri-Beri*, London, 1907. See also: H. Dürk, *Unters. über die path. Anat. d. Beri-Beri*, Jena, 1908.

On the basis of the assumed relationship of a rice diet to Beri-Beri, two principal hypotheses have been brought forward to explain the nature of this relationship. The one hypothesis assumes that a rice diet is physiologically incorrect, owing to the large excess of carbohydrates over nitrogenous and fatty food stuffs¹, whilst according to the other hypothesis Beri-Beri is caused by an unknown poison (alkaloid, ptomaine, toxin) conveyed by and generated in rice by either a mould, microbe, enzyme or some other agency.

Both of these hypotheses have received a certain amount of experimental confirmation, the results of which, however, may be equally well explained by either of the two hypotheses. The main weakness of the "poison" hypothesis consists in the fact that all attempts made to isolate what should be a most intense poison have so far been unsuccessful, whilst the astonishingly beneficial results which have been achieved in Japan by merely replacing a certain percentage of rice by nitrogenous food stuffs², speak strongly in favour of the first mentioned hypothesis, namely, that lack of nutritive material or an unphysiological diet is the cause of Beri-Beri.

In the course of these dietetic changes, introduced first on a large scale in the Japanese navy by Takaki, it was found that the men showed a disinclination to take meat and bread. It was therefore proposed to use barley and rice in equal quantities instead of rice alone. Since the introduction of this change in the navy, not only has Beri-Beri been wholly exterminated but also other general diseases have greatly decreased (Takaki, *loc. cit.*).

The fact that the partial replacement of rice by another cereal like barley is able to prevent the onset of the disease is especially interesting, if we consider the chemical composition of these cereals. It is commonly assumed that the composition of the various cereals only varies within narrow limits and the agreement in the nitrogen percentage of barley and rice, in any case, is very close. According to the analyses given in the report of the Hygienic Laboratory of the Japanese Department of Home Affairs (1897) the average nitrogen percentage of Japanese rice is 1.37 %, whilst that of Japanese barley is 1.82 % (calculated for

¹ It must be mentioned in this connection that, besides containing a relatively small percentage of nitrogen, "white rice" is also very poor in calcium and phosphorus. The lack of these two latter important constituents of a physiological diet, has also been adduced in explanation of the origin of Beri-Beri (see later).

² See also Baron Takaki's lectures, *The Lancet*, Vol. CLXX. 1906, p. 1369. Before the new diet was introduced the number of Beri-Beri cases was 325 per 1000 with 41 deaths annually. With the change of diet the number fell to 127, 6 and 0.4 and finally *nil*.

dried material). König¹ gives 1.52 % N for Japanese rice and 1.55 % N for barley (from Asia).

The beneficial results due to barley, if connected with the nitrogen factor at all, would seem therefore not to be dependent on the amount of nitrogen, but on the nature of the nitrogenous substances.

Until recently nothing was known about the nature of the proteins contained in rice. An investigation carried out by us², furnished the remarkable result that the alcohol-soluble proteins (gliadins³) present in all the known cereals are absent in rice. This fact appears to be of significance in several directions. It explains the impossibility of using rice as a material for making bread. The formation of gluten, the characteristic protein-mixture which enables flour to be converted into dough, is only possible in the presence of a gliadin. The absence of this protein may further conceivably have a bearing on the dietetic value of rice. Gliadins are characterised by the high percentage of glutaminic acid, proline and ammonia amongst their cleavage products. As according to modern views the proteins are broken down completely in the alimentary canal into their cleavage products (mainly amino-acids), before being resynthesised in the body, and as the absence of any one of these amino-acids seems to be intimately connected with the food value of different proteins, it appears justifiable to assume that the absence in rice of such a typical protein as the gliadin, may furnish an explanation of the non-efficiency of the rice diet.

This question can be attacked experimentally by means of feeding experiments on animals. Eykman⁴, and later Grijns⁵, found that an exclusive rice diet produces in fowls a form of polyneuritis, the symptoms, the course and the morbid anatomy of which agree closely with human Beri-Beri. As in the latter, the disease is prevented in fowls if barley, oats or rye are given⁶. These experiments were carried

¹ T. König, *Chem. Zusamm. d. menschl. Nahr. u. Genussm.* Vol. I. 1903.

² The Proteins of Rice, *Proc. Physiol. Soc.* xxxvi. 1908, p. liv. A full communication of this research will appear shortly.

³ The name "gliadin" as a general name for the alcohol-soluble proteins was proposed by O. Rosenheim (*Science Progress*, April, 1908, No. 8) and seems preferable to the term "prolamine" proposed by T. B. Osborne (*Science*, N. S. xxviii. 1908, p. 417) as the resemblance of this name to "protamine" might easily give rise to mistakes.

⁴ *Virch. Arch.* Vol. cxlviii. 1897, p. 523, *Arch. f. Hygiene*, Vol. lviii. 1906, p. 150. We were unable to obtain Eykman's first publication, *Geneesk. Tydschr. v. Ned. Indie*, 1890, in the original.

⁵ *Geneesk. Tydschr. v. Ned. Indie*, 1901, quoted from Eykman. See also: *Arch. f. Hygiene*, Vol. lxii. 1907, p. 128.

⁶ It is also prevented if "unpeeled" or "cured" rice is given, but for reasons stated above these factors need not be considered here.

out in Java and exception has been taken to the results, because they were obtained in a possibly "infected" locality¹.

In order to investigate the question raised by the absence of an alcohol-soluble protein in rice, we carried out feeding experiments on fowls by adding the alcohol-soluble protein of barley (hordein) to their rice diet. In view of the statements that Beri-Beri may be due to the lack of calcium (and a subsequent oxalic acid intoxication²) we also made some experiments in which large quantities of calcium carbonate were added to the food. With regard to the lack of phosphorus it has already been shown by Eykman (*loc. cit.*) that the addition of the organic phosphorus compound contained in rice husks (phytin) is unable to prevent the disease³. It seemed interesting to examine the effect of an inorganic phosphorus compound and we chose calcium phosphate, thus introducing large quantities of calcium and phosphorus at the same time. The effect of these salts was investigated when added to an exclusive rice diet as well as to the rice and hordein diet. Although our experiments are not as numerous as we should wish (owing to the appearance of an infective disease amongst our stock of fowls and also for other reasons) the negative character of the results warrants a short communication.

Description of experiments: Twelve apparently healthy fowls of different breed were used, the birds taken for the corresponding groups of experiments being matched as far as possible in age, weight and breed. They were kept in pairs in separate large wire coops, the floor of which was covered with gravel frequently renewed. The coops were cleaned and disinfected regularly. The birds were weighed at the beginning and at frequent intervals during the course of the experiments. They were allowed exercise daily and were fed, as a rule, twice daily. The fowls fed with barley were allowed to take their food freely. Those fed with rice had to be fed by hand, as they

¹ See, however, Eykman (*loc. cit.*).

² G. Maurer, *Münch. med. Wochenschr.* Vol. LIV. 1. 1907, p. 731. Also *Geneesk. Tydschr. v. Ned. Indie*, 1901, quoted from A. Trentlein, *Verhandl. d. phys. med. Ges. Würzburg*, Vol. xxxviii. 1906, p. 323; see also C. Eykman, *Münch. med. Wochenschr.* Vol. LIV. 1. 1907, p. 127.

³ H. Schaumann (*Verhandl. d. d. tropenmed. Ges.* 1908) ascribes the symptoms of Beri-Beri to the lack of nucleo-phosphoric acid in food. Since this paper has gone to the press an important preliminary communication on this subject has been made by H. Fraser and A. T. Stanton (*Studies from the Inst. f. Med. Research, Fed. Malay States, Kuala Lumpur*). These authors find that the addition to a "white rice" diet of the alcohol-soluble substances (lipoids) extracted from parboiled (cured) rice prevented the development of polyneuritis in fowls and that the estimation of the total phosphorus present in a given rice may be used as an indicator of the Beri-Beri producing power of rice.

soon began to refuse rice food¹. For this purpose the daily portion was powdered and made into pills (the size of a pea) after the addition of a small quantity of water. The animals swallowed these pills easily, when they were introduced a few at a time into their beaks, followed by small quantities of water. All the birds were allowed to take water freely. The rice used was obtained directly from Japan and of the quality usually consumed in that country. On analysis it was found to contain 13.00 % Water, and in the dried material: Nitrogen, 1.24 %, Phosphorus, 0.13 %, Calcium, 0.03 %.

The hordein used in these experiments was prepared from 10 kgs. of barley by extraction with 70 % alcohol. The extracts were concentrated by distillation *in vacuo* and the precipitated hordein purified in the usual way², finally washed with alcohol and ether, and dried *in vacuo* over concentrated sulphuric acid. The amount given with the rice corresponded roughly to that contained in an equal amount of barley. In one set of experiments gluten (*i.e.* a mixture of gliadin and glutenin) from wheat was added to the rice diet.

The following foods were given, two birds being fed on them in each case:

- (1) Rice alone.
- (2) Rice + Hordein (Barley).
- (3) Rice + Gluten (Wheat).
- (4) Rice + Calcium carbonate.
ditto + Calcium phosphate.
- (5) Rice + Hordein + Calcium carbonate.
ditto ditto + Calcium phosphate.
- (6) Barley alone.

Further details are given in the following table.

It will be seen that all the birds fed on rice or on rice and hordein (or gluten) died within the relatively short time of 16—24 days. Death was due in all cases to typical polyneuritis. There was at first general weakness and later evident paralysis, which became usually very marked on the 10th—12th day. The legs seemed affected first, the wings later. The walk was typically unsteady, the birds were unable to sit on the roost and their wings drooped. There was marked loss of sensation

¹ They showed, however, great avidity for barley which they tried to snatch from the other coops, when allowed near them.

² T. B. Osborne, *Journ. Amer. Chem. Soc.* Vol. xvii. 1895, p. 539; see also V. Griessmayer, *Die Proteide der Getreidearten*, Heidelberg, 1897.

to stimuli, dyspnoea, emaciation, and finally death followed general paralysis.

Identical results supervened when calcium carbonate or calcium phosphate was added to the rice diet or to the rice + hordein diet.

No.	Breed	Weight in grammes		Kind of food	Quantity of food	No. of days after which death oc- curred
		Before	After			
1	Buff Orpington	1840	1795	Rice	100 grs. reduced to 50 grs.	16
2	White Leghorn	2005	1720	„	after the 6th day	17
3	Buff Orpington	1930	1750	Rice + Hordein	50grs.Rice, 2·5grs.Hordein	15
4	White Leghorn	1840	1795	„	„	22
5	Plymouth Rock	1865	1405	Rice + Gluten	50 grs.Rice, 2·5 grs.Gluten	22
6	„ „	1708	1508	„	„ „	17
7	Black Minorca	1555	1302	Rice + CaCO ₃	40 grs. Rice, 4 grs. CaCO ₃	14
8	Plymouth Rock	1525	—	Rice + Ca ₃ (PO ₄) ₂	40grs.Rice, 4grs.Ca ₃ (PO ₄) ₂	24
9	Black Minorca	1945	1531	Rice + Hordein + CaCO ₃	40 grs. Rice, 4 grs. CaCO ₃ , 2 grs. Hordein	19
10	Plymouth Rock	1895	1745	Rice + Hordein + Ca ₃ (PO ₄) ₂	40 grs.Rice, 4grs.Ca ₃ (PO ₄) ₂ , 2 grs. Hordein	23
11	Buff Orpington	2247	1498	Barley	Freely	31
12	„ „	2145	1777	„	„	42

In all cases the sciatic and brachial nerves were examined by Marchi's method. They showed distinct and in some cases very marked degeneration (see Plate VI), thus confirming the clinical symptoms and the microscopic *post mortem* changes.

The two control birds fed on barley did not show any of these symptoms. They lived much longer than the other birds, but died unfortunately from an infectious bacterial disease (probably chicken cholera¹), which also attacked and killed rapidly the fowls intended for further investigations. In one of the control animals (No. 11) a number of degenerated fibres were found in the sciatic nerve, although the animal had not shown any paralytic symptoms. A similar observation has also been made by Eykman (*loc. cit.*).

These experiments prove therefore that Beri-Beri, or a disease very similar to it, can be produced in fowls simply by a diet of peeled rice, independently of influences of climate or locality. This fact seems to

¹ Prof. Hewlett kindly examined one of these birds in the Bacteriol. Laboratory, King's College, London. A micro-organism was isolated, which belonged to the group of chicken cholera organisms. We are greatly indebted to Prof. Hewlett for his help in this matter.

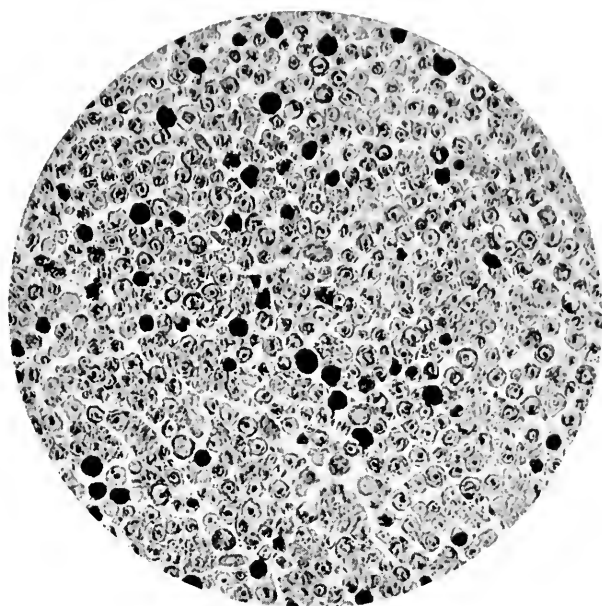


Fig. 1. Photograph showing degeneration of sciatic nerve after rice + hordein diet. Marchi's method. Transverse section.



Fig. 2. Photograph showing degeneration of sciatic nerve after rice + hordein diet. Marchi's method. Longitudinal section.

be of importance for the physiology and hygiene of nutrition, demonstrating the inefficiency of an exclusive rice diet to sustain life.

It would further seem that the alcohol-soluble protein of barley, at any rate in the quantities used, is unable to prevent the disease and that the beneficial action of barley, when added to a rice diet, is due to another constituent of this cereal.

The addition to a rice diet of large quantities of calcium and of phosphorus (as calcium carbonate and calcium phosphate) seems also unable to prevent the disease.

The expenses of this investigation have been in part defrayed out of a grant from the Government Grant Committee of the Royal Society.

ON THE NATURE OF THE CELLULAR ELEMENTS PRESENT
IN MILK. PART II. QUANTITATIVE AND QUALITA-
TIVE RESULTS.

(FOR THE BRITISH DAIRY FARMERS' ASSOCIATION.)

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One Text Figure.

IN a preliminary report (Hewlett, Villar and Revis, 1909, p. 278) published last year a summary of conclusions at which we had arrived with regard to the nature of the cellular elements present in milk was set forth. These conclusions were formulated on results obtained during a prolonged quantitative and qualitative examination of these cellular elements in milks obtained from known sources under veterinary supervision, and possess, we believe, a chronological significance which, with the exception of results published by Russell and Hoffmann (1907), has been somewhat lacking in many investigations. For this purpose we selected four dairy farms at which milk was being produced for sale on ordinary commercial lines, but under various conditions of environment, both architectural and sanitary. Together with these we were also enabled, through the kindness of Professor Percival, to make use of cows in the experimental herd of the University College of Reading.

Our method was to select six cows, generally a month or so after calving, and after careful veterinary inspection of the animals, to have samples of the carefully mixed milk once a week until the cows were so near the end of lactation, that in the ordinary way their milk would

be no longer sold for consumption. As the animals dropped out at varying times we were enabled concurrently to investigate the effect on the cell count, of the termination of lactation. We were thus also in a position to note what warning might be given of the advent of mastitis when it occurred, and what effect on the mixed milk the inclusion of milk from cows suffering from mastitis might have. The farms being run on commercial lines we also knew to what extent the milk of cows suffering from mastitis would be included in a general supply, it being the common practice of farmers to include milk so long as it is unchanged in appearance and derived from quarters of the udder not apparently affected by any diseased condition. This practice, though often condemned, is in our opinion probably without danger to the community, except in certain special cases to which we shall refer.

The term "mastitis" includes all forms of inflammatory disease of the mammary gland. It may be interstitial or catarrhal, acute, sub-acute, or chronic, localised to a small portion of one quarter of the gland or involving a quarter, quarters, or the whole of the gland. It varies in intensity from a slight, transitory and hardly perceptible condition to one in which there is a considerable swelling and thickening, tenderness, local heat and general constitutional disturbance and fever. Definite suppuration with abscess formation seems to be rare. In a definite catarrhal mastitis the normal secretion is replaced by a yellowish serous fluid.

The slighter forms of so-called mastitis are probably very common and may arise from slight injuries, or even careless milking. The effect on the cell count, as we believe we show, seems to be as marked in the slight and transitory cases, in which the condition is revealed only by a careful examination, as in the more severe and obvious cases.

We also regard the character of the secretion as indicative of the severity of the condition. It must be clearly understood that when the term "mastitis" is used in this paper there was not the slightest indication of even a trace of suppuration in any case.

It is to be noted that it is quite common in the early period of lactation for the breasts of the suckling woman to become unequally swollen, knotty and painful. This is ascribed to obstruction in the lacteal ducts preventing a free outflow of the secretion. In the severer cases there may be general constitutional disturbance, thickened lymphatics and enlargement of the axillary glands. The condition almost always ends in resolution. It seems very probable that a similar condition may obtain in the cow and constitute these mild forms of so-called mastitis, especially that termed "interstitial mastitis."

We have also investigated in a similar manner the effect of the commencement of lactation, but have been forced to leave the consideration of the effects of feeding, and also of the microscopical structure of the udder tissue itself, to a further report.

The number of cows selected, viz. six, may at first sight seem too small to represent practical conditions, but in our opinion milk is constantly sold from such a small number, and further, if the number be large, the work becomes unwieldy, and the supervision not sufficiently rigid; and moreover, in such a small herd, the inclusion of one abnormal animal would produce a more marked and noticeable effect than if the number of animals were large.

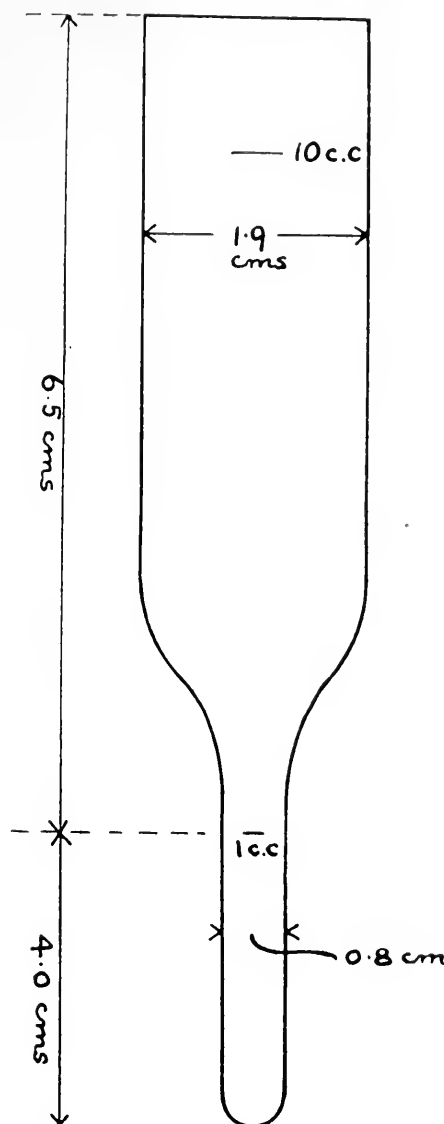
We have attempted to approach our results in a frame of mind unbiassed by prevailing ideas, so that the conclusions at which we have arrived regarding the nature of these cellular elements seem to compel a reconsideration of the causal relationship of streptococci to mastitis, and we hope shortly to make this the subject of experimental investigation.

I. The quantitative examination of milk for cellular elements.

For quantitative examination we have adopted a uniform method which is here described:

Early in the investigation our attention was directed to the work of Russell and Hoffmann (1908), who have shown that by heating milk to 60°—70° C. for 10 minutes before centrifugalisation, a very great increase in the number of cells takes place, and they attribute this to an effect on the fat globules, which being broken down from their cluster formation by the heat cease to have an entangling effect which normally results in the carrying of cells to the surface, and a much larger deposit is therefore obtained. This work has been amplified by Campbell (1909, p. 15), who not only confirms the work of his predecessors, but concurs in their conclusion as to the action of heat. We also concur in the action of heat as an agent increasing the number of cells, but differ as to the explanation.

Before Russell and Hoffmann's work had come into our hands we had found that the addition of about 6 drops of formalin (40 % formaldehyde) to 60—70 c.c. of milk had the effect of greatly increasing the number of cells if the milk be allowed to stand 24 hours. We have compared this method with the "heating" method and find that within the limits of error of counting, the two methods give very similar results. There is a further advantage in the use of formalin, viz. that this liquid can be introduced into milk the moment it is obtained from the cow, and the



In a tube of the shape and dimensions shown in the figure are placed 5 c.c. of the well mixed milk, diluted to the 10 c.c. mark with 0.8 % salt solution. After inserting a rubber stopper the contents are well mixed. The tube is then centrifuged at about 2000 revs. per min. for two minutes, the cream is broken up by violently shaking the upper part of the tube and the rotation continued for four minutes longer. A glass rod, fitting roughly the narrow neck of the tube, is inserted and the major part of the milk poured off and the upper part of the tube well rinsed with water to remove cream, etc.; the contents of the narrow end down to within $\frac{1}{4}$ inch of the deposit are sucked out with a fine glass pipette, the upper part of the tube wiped clean, and the tube then filled to the 10 c.c. mark with salt solution. The tube, having been violently shaken till all the deposit is distributed through the liquid, is then rotated for four minutes and the liquid down to within $\frac{1}{4}$ of an inch of the deposit again removed. In the case of small deposits 2 to 3 drops of saturated aqueous solution of methylene blue are added, and the deposit stirred up by blowing through a fine glass capillary pipette (which is afterwards used for filling the counting chamber). After 15 minutes, water is added to the 1 c.c. mark and counting done in the usual way with a Thoma-Zeiss blood-counter. Counting should not be restricted to the ruled spaces, but the field so arranged that a definite number of squares is included and fields counted all over the chamber. At least two different preparations should be made of the same deposit for counting.

In the following examinations, the field was so arranged that its diameter was 8 small squares of the counter (Leitz-Thoma-Zeiss); then if n be the number of cells per field:

$$n \times 16,000 = \text{number of cells per c.c.}$$

or the method proposed by Savage (1906, p. 127) may be adopted.

sample used after 24 hours without further manipulation, and at the same time the cells themselves are preserved without alteration for microscopical examination.

Now it is evident that there can be no action on the fat globule clusters in this case, such as does occur to a certain extent when milk is heated to 60°—70° C., and we must look for another explanation. One of the most striking effects of formalin or heat is the disruptive action on clusters of the cells themselves, and it would seem more probable that these agents break down aggregations of cells, perhaps by destroying some agglutinative property present, and by thus producing a more even distribution of these cells cause an apparent numerical increase. As a temperature of 70° C. and formalin both have a destructive effect on enzymes and substances of similar nature, this explanation seems reasonable.

The carrying power of the fat globules not only for cells, but also for blood, bacteria, etc. is undoubted, and it may well be that some attractive force between the fat globules and the cells is also broken down by heat or by formalin.

The formalin method has been used throughout this investigation (with one exception), and this must be taken into account in considering the figures given.

Should the deposit for counting be large, a correspondingly larger amount of methylene blue must be added and the whole diluted to 5, 10, or more cubic centimetres, as may be required.

*A preliminary experiment to determine the distribution
of cells in the milk stream.*

A number of counts were made of milk drawn from one quarter of a cow in successive portions in order to determine whether these cells appeared uniformly during the process of milking or not. The results obtained are tabulated on the opposite page.

From these results it is fairly evident that the cells appear practically uniformly throughout the milking, the slight increase in the strippings being probably caused by increased manipulation of the udder by the milker in stripping. It seems safe to assume that these cells pass into the milk regularly during its elaboration in the alveoli and are not a response to any stimulus given to the tissues by the process of milking. They do not therefore appear to be connected with any particular constituent of the milk, but to be the result of the general activity of the gland tissue.

		No. of cells per c.c.	Quantity of milk in c.c.	Fat	T. solids
Exp. I.	Fore milk	17,000	170	3.95	13.64
		40,000	340	4.60	14.56
		31,000	284	5.60	15.20
	Strippings	32,000	284	6.30	16.01
Exp. II.	Fore milk	155,000	260	1.80	11.12
		155,000	300	2.52	11.80
		165,000	300	3.20	12.32
	Strippings	230,000	170	4.00	13.02
Exp. III.	Fore milk	24,000	150	0.80	10.24
		20,000	150	0.70	10.18
		30,000	170	0.70	10.16
	Strippings	20,000	240	1.42	10.72
Exp. IV.	Fore milk	30,000	200	2.22	11.86
		32,500	400	2.60	12.24
		30,000	450	2.40	12.03
	Strippings	70,000	460	4.37	13.76
Exp. V.	Fore milk	147,000	160	1.90	10.48
		137,000	430	3.00	12.12
		145,000	240	3.05	12.00
	Strippings	290,000	130	4.45	13.16
Exp. VI.	Fore milk	5,300	330	0.68	10.44
		4,700	990	0.55	10.31
		2,200	415	0.60	10.33
	Strippings	1,500	460	0.62	10.32

In general, in the case of any particular cow, apart from changes produced by some special causes, the number of cells per c.c. is fairly constant over the lactation period, from which we may infer that these cells are closely connected with milk production, though no light is thus thrown upon their character. As however we shall see that in general in the case of cows which are in calf there is a large increase of these cells at the end of lactation, while in the case of cows which are barren, no such increase usually takes place, there seems a certain amount of support to the view that these cells are tissue cells, as in the former case we should expect a considerable regenerative action to be taking place in the gland tissue, but not in the latter.

DAIRY FARM A.

To determine the effect of the introduction of newly calved cows on the cell count of the mixed milk.

Six cows were selected and at intervals two were dropped out and two newly calved cows introduced in their place. The results were as follows:

(The counts in this experiment were done without the use of heat or formalin, the numbers are therefore lower than would otherwise have been obtained.)

No. of cows	Date	No. of cells per c.c.	Remarks
Six	17. 11. 08	670,000	—
„	25. 11. 08	287,000	—
„	1. 12. 08	278,000	—
„	9. 12. 08	384,000	—
„	17. 12. 08	261,000	One dropped out and one newly calved in
„	18. 12. 08	287,000	—
„	23. 12. 08	705,000	—
„	31. 12. 08	230,000	—
„	6. 1. 09	—	Two out and two newly calved in.
„	12. 1. 09	250,000	—
„	19. 1. 09	587,000	—
„	26. 1. 09	205,000	—
„	3. 2. 09	11,000	—
„	9. 2. 09	220,000	—
„	19. 2. 09	511,000	Two out and two newly calved in.
„	24. 2. 09	272,000	—
„	30. 2. 09	220,000	—
„	8. 4. 09	572,000	Two out and two newly calved in (one
„	13. 4. 09	237,000	three days only since calving).
„	20. 4. 09	697,000	—
„	28. 4. 09	412,000	(The two new cows were examined sepa-
„	5. 5. 09	798,000	rately, see below.)
„	11. 5. 09	1,528,000	—

On 28. iv. 09 the milks of the two last cows were separately examined, (1) because the number of cells had increased suddenly the week before, and (2) because the milker considered that the milk of one of the cows had become slightly ropy, though there was no evidence of this in the samples received by us.

The results of the examination were as follows:

Cow I.	28. iv. 09	1,390,000 cells per c.c.
Cow II.	„	90,000 „ „

Analyses.

Cow I.	Total solids	12.30 %	Fat	3.50 %.
Cow II.	„ „	13.14 %	„	4.20 %.

The milk of Cow I was undiminished in quantity and was not in the least abnormal in appearance.

On May 13 this cow (No. I) was examined by Mr Villar, who gave the following report :

“ I examined the cow in Kent yesterday. She is a good sort of Shorthorn and had her second calf about six weeks ago.

I am told that at her first calving she had mastitis affecting the right front quarter of her udder—that quarter is now atrophied and has lost its functional activity, so that we have not been receiving milk from that particular quarter.

There is a slight sub-acute interstitial mastitis of the right hind quarter, following, I should think, a more acute process.

The milk from the three quarters had the appearance of normal milk:—her afternoon yield was 12 lbs.”

The cowman had however noticed no signs of inflammation, or abnormality of the udder, which would point to a more acute process.

The milk of this cow was examined several times during the next two months. Except in the case of the first two and the fifth samples the milk samples of the two sound quarters were mixed, and that from the suspected (R. H.) quarter examined separately. The milk was also analysed more or less completely, in order to ascertain the effect of the high cell output on the quality.

The results were as follows :

Date		No. of cells per c.c.	Analyses		
			Fat	T. solids	
6. 5. 09	Mixed milk	5,110,000	3.60	12.18	
11. 5. 09	„	5,850,000	3.50	12.06	
18. 5. 09	(2 sound quarters)	2,905,000	3.20	11.87	Lactose 4.62
„	(R. H. quarter)	3,632,000	3.30	12.18	Lactose 4.48
21. 5. 09	Mixed milk	4,485,000	3.75	12.52	
27. 5. 09	(2 quarters)	3,700,000	3.45	12.25	
„	(R. H. quarter)	10,000,000	3.20	12.10	
4. 6. 09	(2 quarters)	3,710,000	2.40	11.40	
„	(R. H. quarter)	5,125,000	3.25	12.08	Lactose 4.45
9. 6. 09	(2 quarters)	1,656,000	3.30	12.06	
„	(R. H. quarter)	3,242,000	3.65	12.60	
15. 6. 09	(R. H. quarter)	2,995,000	2.95	12.04	
17. 6. 09	(2 quarters)	2,280,000	3.95	12.87	

The cell count which was at first very much higher in the suspected quarter, gradually fell till the quarters were eliminating cells fairly

evenly, though the total count remained very high. There was not, at any time, any abnormal appearance of the udder to the ordinary observer.

Two months later (23. VIII. 09) the milk from this cow was again examined with the following results:

(2 quarters)	5,280,000 cells per c.c.
(R. H. quarter)	3,120,000 „ „

Analyses of above:

	Fat	T. S.	N. F. S.	Lactose	Protein & Ash
(2 quarters)	4.20	13.08	8.88	4.26	4.62
(R. H. quarter)	3.75	12.26	8.51	4.26	4.25

The cell content of the milk of the two sound quarters now exceeded that of the so-called affected quarter. The milk was quite normal chemically, and the high count of cells was not in the case of this cow accompanied with an abnormally depressed percentage of lactose, which, as we shall see, is often to be observed in such cases. This case is very interesting, as we have here a cow which has a slight and transitory affection of one quarter of the udder, producing for a long time a large number of cells from the udder, but without any change in the milk secretion either in quantity or quality, showing that the vital activity of the gland tissue was in no way affected. There is no evidence to show that such milk is injurious; yet if a cell count be relied upon, such milk might at any time have been supposed to be the product of a cow or cows suffering from severe mastitis. Reference will be made to this cow again in our general conclusions.

DAIRY FARM B.

Six cows were selected here and carefully examined. The individual cell counts of these cows at the start, and their dates of calving, were as follows:

Ref. No. of Cow	Age	Last calving	Cells per c.c.
25	5 years	Feb. 12, 1909	14,000
26	6 „	Mar. 1, „	533,000
27	5 „	Jan. 6, „	264,000
28	5 „	Mar. 7, „	75,000
29	4 „	Feb. 14 „	12,500
30	6 „	Mar. 4 „	32,500

Their usual feed consisted of a mixture of chaff, grains, flaked maize, bran, bean meal, and half a bushel of mangels a day, and as much hay

as they could eat with about 4 lbs. of linseed and Waterloo cake mixed. The first milk was received on April 7th, 1909, and the samples continued till the cows were dry.

The weekly results were as follows :

No. of Cows	Date	No. of cells per c.c.	Remarks
Six	7. 4. 09	82,000	—
„	13. 4. 09	157,000	—
„	20. 4. 09	120,000	—
„	27. 4. 09	367,000	—
„	4. 5. 09	456,000	Cows turned out to grass after this sample.
„	11. 5. 09	115,000	—
„	18. 5. 09	256,000	—
„	25. 5. 09	142,000	—
„	1. 6. 09	315,000	—
„	8. 6. 09	161,000	—
„	15. 6. 09	667,000	—
„	22. 6. 09	329,000	—
„	29. 6. 09	726,000	—
„	6. 7. 09	467,000	—
„	14. 7. 09	725,000	—
„	27. 7. 09	421,000	The cows were examined and found healthy
„	4. 8. 09	458,000	by Mr Villar, one (27) considered to be far in calf.
„	7. 8. 09	—	Individual counts made again with follow- ing results :

Ref. No. of Cow	No. of cells per c.c.
25	35,000
26	1,410,000
27	3,545,000
28	128,000
29	32,000
30	100,000

The cows were examined and 27 reported quite healthy, but one quarter (L. F.) of 26 seeming slightly abnormal she was dropped out after the next sample and her milk was examined separately (see below).

Six	11. 8. 09	770,000	—
Five	18. 8. 09	1,075,000	—
(Nos.25,27,28,29,30)			
„	20. 8. 09	—	Cow 28 reported to have a bad quarter
Four	25. 8. 09	829,000	(L. H.). She was dropped out.
(Nos.25,27,29,30)			
„	31. 8. 09	240,000	—
„	7. 9. 09	207,000	—
„	15. 9. 09	557,000	—

No. of Cows	Date	No. of cells per c.c.	Remarks
Four			
(Nos. 25, 27, 29, 30)	23. 9. 09	1,210,000	Nothing to account for these fluctuations.
"	6. 10. 09	295,000	
"	12. 10. 09	1,735,000	
"	19. 10. 09	250,000	
"	20. 10. 09	—	Cow 27 gave abnormal milk from one quarter. Not diseased, but cow drying off. She was dropped out.
Three	27. 10. 09	445,000	Higher than last week with cow 27 in.
(Nos. 25, 29, 30)	3. 11. 09	44,000	—
"	9. 11. 09	46,000	—
"	16. 11. 09	42,000	Cow 30 dried off.
Two	23. 11. 09	22,000	—
(Nos. 25, 29)	30. 11. 09	62,000	—
"	7. 12. 09	17,000	—
"	17. 12. 09	26,000	—
"	21. 12. 09	133,000	—
"	28. 12. 09	22,000	—
"	4. 1. 10	22,000	Cows dried off.

These cows gave some very remarkable results. In general it must be noted that the end of lactation with the three cows—Nos. 25, 29 and 30—had no effect at all in raising the cell count and they were all barren. Cow 27 was a very disturbing factor, and while at no time showing any signs of disease gave very large cell counts towards the end of lactation, and it is evident from the figures obtained between 15. IX. 09 and 20. X. 09, that this cell count was of a very fluctuating nature. Below are given some further details of this cow.

On 7. VIII. 09 there was apparent evidence that Cow 26 might be developing mastitis, which however did not occur; the only disturbance being a diminished quantity of milk from her L. F. quarter. On the other hand Cow 28, which on 7. VIII. 09 gave no evidence at all of anything wrong, by 20. VIII. 09 had developed a severe mastitis in her L. H. quarter, so that the cell count was of no premonitory value and the onset very sudden. A similar case is referred to later.

Further Examination of Cow 26.

This cow was reported on 7. VIII. 09 as having a very slight abnormal appearance of the L. F. quarter. It was not sufficient to diagnose any definite disease, but the milk from this quarter was diminished in quantity. Cell counts from each quarter were made as follows:

Date		Cells per c.c.	Remarks
19. 8. 09	R. H.	200,000	All samples quite normal in appearance.
	L. H.	356,000	
	L. F.	6,960,000	
	R. F.	424,000	
25. 8. 09	R. H.	736,000	The quantity of milk from L. F. quarter was still small in quantity.
	L. H.	252,000	
	L. F.	8,840,000	
	R. F.	2,292,000	

Analysis of milk of L. F. quarter :

Fat 1·27 %, T. Solids 8·18 %, N. F. S. 6·91 %, Lactose 2·22 %, Protein & Ash 4·69 %.

The milk is abnormal only as regards lactose, a very common occurrence when low solids are found, and generally with a high cell count.

Date		Cells per c.c.
7. 9. 09	R. H.	64,000
	L. H.	128,000
	L. F.	10,640,000
	R. F.	372,000

This cow is a very good example of one giving a persistent high cell count with but slight and transitory cause, and the milk was never changed in appearance.

Further Examination of Cow 27.

This cow on 7. VIII. 09 gave a very high count in her mixed milk. She was reported by the veterinary surgeon as drying off rapidly being far in calf, but she did not become dry till 20. x. 09, when she dried off with great suddenness, and calved on 28. XII. 09 in a normal manner.

Cell counts and analyses of the milk from each quarter were made with the following results :

Date		No. of cells	Remarks
10. 9. 09		3,920,000	Mixed milk.
14. 9. 09	R. H.	7,200,000	
	L. H.	940,000	
	L. F.	6,800,000	
	R. F.	2,240,000	

Analysis :

	Fat	T. solids	N. F. S.	Lactose	Protein & Ash
R. H.	3·15	11·36	8·21	3·64	4·57
L. H.	2·80	11·98	9·18	4·50	4·68
L. F.	3·40	12·20	8·80	3·98	4·82
R. F.	2·40	11·78	9·38	4·58	4·80

The diminished sugars correspond to the high cell counts.

This cow on 20. X. 09 gave abnormal fluid from one quarter (R. H.), but not caused by disease. The R. F. and L. F. quarters gave very large deposits, but were not counted. The milk from these three quarters (R. F., L. F. and L. H.) was quite normal in appearance and would have been put in with other milk and undoubtedly have caused a very large cell count in the mixed milk. Each of the fore quarters would have contributed at least 80,000,000 cells per c.c.

The milk of this cow was again examined on 6. I. 10, nine days after calving, when the number of cells per c.c. was 3,340,000, so that the high cell count continued. The milk of each quarter was examined on 22. I. 10 with the following results:

	No. of cells per c.c.
R. H.	36,000
L. H.	360,000
L. F.	6,200,000
R. F.	5,200

It will be noted that the high cell count is continued after calving in the L. F. quarter, but in the R. H. quarter has fallen to a very small number. This is a remarkably good instance of the continuity of cell proliferation in quarters which have already given high counts, in spite of the regenerative tissue changes which presumably have taken place.

Cow 28.

This cow developed about 18. VIII. 09 a severe catarrhal mastitis of the L. H. quarter. The milk of each quarter was examined on 23. VIII. 09.

L. H.	Yellow watery liquid.
L. F.	452,000 cells per c.c.
R. F.	282,000 ,,
R. H.	1,470,000 ,,

It will be observed that the addition of the milk of the unaffected quarters to other milk would not materially affect the total cell count, and would therefore give no evidence that milk from a diseased cow had been added.

It is true that the week before this cow developed mastitis the total cell count of the six cows (q.v.) rose to over 1,000,000 per c.c., but as this number was exceeded on 23. IX. 09 and 12. X. 09 when the mixed milk was from cows not diseased, the indicative value of the cell count

is doubtful. From other observations it seems probable that there is no rise in the number of cells in the milk of a cow about to develop mastitis until a day or two before the affection is visible to the eye.

DAIRY FARM C.

(These cows were not examined by Mr Villar, but by the Veterinary Surgeon attached to the Farm.)

This farm is used for the production of milk for nursery use and only high-class tuberculin-tested animals are stalled. The feed is of rather a rich character including locust and bean meals, etc. The cows are kept in large, first class sheds, fitted with every up-to-date requirement, and they are under constant veterinary supervision.

The results are extremely interesting, as there was not the least trace of disease at any time in the shed ; while the cell counts obtained were often extremely high.

Six cows were selected as usual and carefully examined. They averaged about five years old and were all Shorthorns.

An individual count at the commencement gave the following results :

Ref.no. of Cow	Date	No. of cells per c.c.
1	21. 4. 09	75,000
2	„	151,000
3	„	14,000
4	„	78,000
5	„	9,500
6	„	70,000

Weekly samples were then taken as usual :

Number of cows	Date	Cells per c.c.	Remarks
Six	5. 5. 09	322,000	—
„	12. 5. 09	136,000	—
„	18. 5. 09	295,000	—
„	26. 5. 09	635,000	—
„	2. 6. 09	478,000	—
„	9. 6. 09	303,000	—
„	16. 6. 09	563,000	—
„	23. 6. 09	343,000	—
„	30. 6. 09	357,000	—
„	7. 7. 09	385,000	—
„	14. 7. 09	740,000	—
„	28. 7. 09	671,000	—
„	4. 8. 09	1,186,000	Cows reported quite healthy by veterinary surgeon.
„	11. 8. 09	471,000	

Cellular Elements in Milk

Number of cows	Date	Cells per c.c.	Remarks
Six	14. 8. 09	—	Individual counts were made again :
		Ref. no. of cow	Cells per c.c.
		1	1,745,000
		2	2,300,000
		3	68,000
		4	198,000
		5	462,000
		6	643,000
		Cow 3 was drying off and was dropped out here. All were reported quite healthy by the veterinary surgeon.	
Five	18. 8. 09	817,000	Cow 6 stated to be giving very slightly less milk from one quarter (L. H.).
(No. 3 dropped out.)			The milk of Cow 6 was examined (see below). The veterinary surgeon reported as follows :—"Has a slight thickening in the near hind quarter of udder. Temp. 102·2°, pulse normal. She is quite healthy." N.B.—This thickening referred to disappeared practically entirely before the end of lactation. The lessened quantity of milk was only ephemeral, as eight days later each quarter was giving practically the same quantity when actually measured.
			Cows 1 and 2 were reported quite healthy.
	25. 8. 09	640,000	Including Cow 6 which had recovered her quantity in L. H. quarter.
"	31. 8. 09	1,678,000	
"	8. 9. 09	1,450,000	—
"	15. 9. 09	1,070,000	—
"	23. 9. 09	660,000	—
"	29. 9. 09	815,000	—
"	7. 10. 09	1,580,000	—
"	13. 10. 09	1,635,000	—
"	20. 10. 09	650,000	—
"	27. 10. 09	669,000	Cow 1 dropped out here.
Four	4. 11. 09	1,680,000	—
"	10. 11. 09	330,000	—
"	16. 11. 09	786,000	—
"	24. 11. 09	1,020,000	—
"	1. 12. 09	4,255,000	Cows reported quite healthy.
"	3. 12. 09	—	Individual counts were again made with the following results :
		Ref. no. of Cow	Cells per c.c.
		2	3,900,000
		4	644,000
		6	1,000,000
		7	460,000
		Cow 2 was very carefully examined and was found perfectly healthy in every way.	
		Cow 6 is still contributing a high cell count.	
		The cows are of course nearing the end of lactation, but still milking well.	

Number of cows	Date	Cells per c.c.	Remarks
Four	8. 12. 09	778,000	Note the drop in the cell count.
„	15. 12. 09	981,000	—
„	21. 12. 09	519,000	—
„	29. 12. 09	985,000	—
„	5. 1. 10	1,982,000	—

Further Examination of Milk of Cow 6.

The milk of this cow was carefully examined on several occasions and also analysed with the following results :

Date	No. of cells per c.c.
19. 8. 09	R. H. 7,210,000
	L. H. 540,000
	L. F. 112,000
	R. F. 84,000

Analysis :

	Fat	T. Solids	N. F. S.	Sugar	Protein & Ash
R. H.	2·10	10·80	8·70	4·3	4·4
L. H.	1·50	9·90	8·40	4·1	4·3
L. F.	2·25	10·66	8·41	4·2	4·2
R. F.	2·85	11·44	8·59	4·2	4·4

None of this milk was at all abnormal in appearance.

Date	No. of cells per c.c.	Date	No. of cells per c.c.
26. 8. 09	R. H. 5,360,000	29. 10. 09	R. H. 1,320,000
	L. H. 372,000		L. H. 256,000
	L. F. 120,000		L. F. 600,000
	R. F. 136,000		R. F. 300,000
10. 9. 09	R. H. 1,940,000	18. 1. 10	R. H. 920,000
	L. H. 1,040,000	(3 months	L. H. 1,496,000
	L. F. 318,000	later)	L. F. 1,800,000
	R. F. 152,000		R. F. 2,680,000

The results here are very curious as the R. F. quarter now gives the high count. The cow is nearly dry.

The milk of this cow is most interesting, as it is a good example of very high cell counts without satisfactory cause. The veterinary surgeon could give no explanation of the slight thickening of the one quarter, and there was no evidence at all of any diseased condition. There is no doubt that this cow gave fluctuating, and often very high, counts from the R. H. quarter, and was responsible for the high count experienced at times in the mixed milk, though there is no doubt that Cow 2 also contributed heavily at times. This cow was slaughtered on February 14th, 1910, and portions of the udder used for microscopical examination, the results of which will appear in a subsequent report.

Further Examination of Milk of Cow 2.

Date		No. of cells per c.c.
6. 12. 09	R. H.	1,112,000
	L. H.	21,200,000
	L. F.	776,000
	R. F.	2,800,000
10. 12. 09	L. H.	3,880,000

Analysis of milk from L. H. quarter :

Fat 3.35, T. Solids 12.22, N. F. S. 8.87, Lactose 3.82, Protein & Ash 5.05.

Note high protein and low sugar as usual with the high cell count.

Date		No. of cells per c.c.
31. 12. 09	R. H.	212,000
	L. H.	352,000
	L. F.	616,000
	R. F.	500,000

The milk of this cow was not diminished at any time, nor did the udder at any time present any abnormal appearance, nor could the least hardening be detected. There was no doubt that she was in perfect health. It will be noted that on 31. XII. 09 the milk showed a fairly normal cell count from each quarter.

Milk of Cow 3 analysed 23. 8. 09, when nearly dry.

Fat 4.00, T. Solids 13.83, N. F. S. 9.83, Lactose 4.08, Protein & Ash 5.75.

The protein and ash are abnormally high, but the milk was quite normal in appearance and taste.

There was no particular reason for examining this milk, as the cow was on 14. VIII. 09 giving only a small cell count, but it is interesting from the fact that the composition is practically the same as that from udders giving high cell counts.

DAIRY FARM D.

To determine the effect of calving on the cell content of the milk.

For this purpose cows were selected which had calved from one week to a fortnight before the samples were taken. We were not concerned with the actual immediate effect of parturition, but only with any effect that might be produced in milk sold for consumption,

for which purpose an interval of about a week is usually allowed. The following method was adopted:

Starting with one cow, samples were taken weekly from her, till another newly-calved cow was available when the mixed milk of these two was examined, and so on, till six newly-calved cows had been brought into use.

Number of cows	Date	No. of cells per c.c.	Remarks
(No. 33)	26. 10. 09	339,000	—
(No. 34)	3. 11. 09	110,000	Contained a trace of blood.
(Nos. 33 & 34)	4. 11. 09	279,000	„ „ „
(No. 35)	10. 11. 09	36,000	—
(Nos. 33, 34 & 35)	„	138,000	—
(No. 36)	17. 11. 09	415,000	—
(Nos. 33, 34, 35 & 36)	„	3,290,000	—
	22. 11. 09	—	The cows were examined separately with the following results:

Ref. no. of cow	Cells per c.c.
33	352,000
34	56,000
35	136,000
36	1,760,000

(See below.)

(No. 38)	24. 11. 09	99,000	—
(Nos. 33, 34, 35, 36, 38)	„	483,000	—
	26. 11. 09	—	34 suffering from severe catarrhal mastitis in L. H. quarter. She was dropped out. 36 had a circumscribed swelling on R. H. quarter, but milk not affected.
(No. 37)	1. 12. 09	4,360,000	—
(Nos. 33, 35, 36, 38, 37)	„	1,295,000	—
	3. 12. 09	—	37 was carefully examined. Mr Villar reported:

“ She is a shorthorn of good class and had calved 11 days, was giving 16 quarts of milk per day and appears in very good health, but her temperature was one degree above normal.

She has what is known as a ‘fleshy’ udder —this is quite a normal condition, but in this cow there is an abnormality of right hind quarter, viz., it is slightly larger and the least bit more firm to the feel than the corresponding quarter—there is no pain or increased local temperature. Milk from it appears normal in quality and quantity. The condition is not observable, except on very careful examination.”

The cowman stated that ‘she ran her milk’ in the morning from the L. H. quarter. She was giving full normal milk from *all* quarters.

Number of cows	Date	No. of cells per c.c.	Remarks
1. (No. 34 A, instead of old 34)	8. 12. 09	730,000	—
6. (Nos. 33, 34 A, 35, 36, 38, 37)	„	885,000	—
	15. 12. 09	1,073,000	—
	22. 12. 09	1,174,000	—
	24. 12. 09	—	Mr Villar examined 36 and 37 and reported as follows:
<p>“ Cow No. 37. The right hind is now quite normal, but the left hind quarter is obviously swollen throughout, the milk appears normal and the quantity from this quarter does not differ from that given from the other quarters, but there was at this afternoon’s meal (24th inst.) a considerable <i>total</i> falling off from that at my previous visit. The cow’s temperature was 102·6, and she has fallen away in condition and her appetite is not very good. I should regard it as a non-specific interstitial mastitis.</p> <p>Cow No. 36 had also a marked <i>local</i> mastitis at the upper posterior part of the right hind quarter—there is no external sign of injury, although the symptoms rather suggested that cause. It was semi-acute and may go on to suppuration. The milk was not altered in appearance, and did not appear to be in quantity either from this quarter—cow in herself quite well—no sign of tuberculosis. I do not think that the actual secreting tissue is affected by the swelling.”</p>			
6. (Nos. 33, 34 A, 35, 36, 38, 37)	29. 12. 09	558,000	—
Ditto, (but not including milk from L. H. of 37.)	5. 1. 09	1,364,000	—

As sufficient samples had been examined, this supply was not continued further.

The interpretation of the above results is very difficult, as it is much complicated by the appearance of mastitis, or other udder affection, in the cows. Some points however are very noteworthy.

The original Cow 34 is the only one which developed a typical catarrhal mastitis and it is to be noted that she gave *no* premonitory symptoms as regards alteration in the count. On 17. XI. 09 the very large count rather pointed to some such trouble, but on 22. XI. 09 the cell count of this cow was only 56,000. Two days later, she had a severe mastitis of the L. H. quarter, from which milk ceased and was replaced by the usual yellowish watery fluid. The veterinary surgeon reported that it was of some days’ standing probably, but two days previously there was no evidence of the disease as indicated by the cell count.

On 30. XI. 09 each quarter was examined with the following results :

R. H.	64,000 cells per c.c.
L. H.	Yellow watery fluid.
L. F.	100,000 cells per c.c.
R. F.	20,000 ,, ,,

The noteworthy point here is the low cell counts in the unaffected quarters. This is of very great importance, as *in practice the farmer would milk the L. H. quarter on the ground and mix the milk of the other quarters with other milk*, and there would not be the least indication that the milk was not from a healthy cow. The inadequacy of cell counts to detect or foreshadow mastitis in certain cases is well exemplified in the case of this cow.

She did not develop any disease of these other three quarters within the time of our experiment.

Cow 37.

The milk of all four quarters of this cow was examined on 13. XII. 09 with the following results :

R. H.	73,000,000 cells per c.c.
L. H.	(Sample broken.)
L. F.	11,240,000 cells per c.c.
R. F.	1,800,000 ,, ,,

The milk was quite normal in appearance from all the quarters examined, but the deposit from the R. H. quarter contained quantities of long chain streptococci. Some of this deposit was injected into a young rabbit, but no ill effects at all followed.

On 17. XII. 09 the milk from these four quarters was analysed :

	Fat	T. Solids	N. F. S.	Lactose	Protein & Ash
R. H.	3.55	11.64	8.09	2.98	5.11
L. H.	3.90	12.62	8.72	4.74	3.98
L. F.	3.90	12.76	8.86	4.95	3.91
R. F.	3.25	12.14	8.89	5.01	3.88

All these milks were quite normal in appearance, and the low sugar in the case of milk of the R. H. quarter, which gave such a heavy cell count, is again to be noted. Here it is accompanied by a rise in protein.

Seven days later this R. H. quarter was quite normal (in fact, it had only been slightly abnormal in appearance from the first), but the L. H.

quarter was now swollen as noted above, and on 30. XII. 09 a sample of milk from each quarter was again examined :

R. H.	84,000,000 cells per c.c.
L. H.	79,000,000 " "
L. F.	684,000 " "
R. F.	1,148,000 " "

The milks were all quite normal in appearance, but the deposits both from the milk of the L. H. and R. H. quarters contained numbers of long chain streptococci.

The milk was again examined on 20. I. 10 :

R. H.	Uncountable (100,000,000 approx.) cells per c.c.
L. H.	" (" ") " "
L. F.	6,440,000 cells per c.c.
R. F.	2,820,000 " "

The deposits from the R. H. and L. H. quarters were full of streptococci in thick masses. The same organisms were also present in the two fore quarters. A large amount of deposit from the milk of each of the two hind quarters was injected into two young rabbits. In both cases no ill effects resulted. It is to be particularly noted that she ran her milk from both hind quarters, because if the sphincter muscle was not sufficiently strong to stop the egress of milk, it could not stop the ingress of streptococci.

The milk of the four quarters was analysed on 27. I. 10 with the following results :

	T. Solids	Fat	N. F. S.	Lactose	Protein & Ash
R. H.	9.76	2.40	7.36	2.18	5.18
L. H.	10.41	2.80	7.61	2.82	4.79
L. F.	13.40	4.30	9.10	4.42	4.68
R. F.	11.72	2.55	9.17	5.00	4.17

In the milk of the R. H. and L. H. quarters the lactose is much depressed and protein high. The milk of the hind quarters was very slightly brownish, but only noticeable in comparison with other milk.

Mr Villar on 19. I. 10 reported as follows :

"Cow No. 37. Right hind quarter normal. Left hind quarter, mastitis slightly more marked than at my previous visit, quarter somewhat harder, but not any larger, first milk drawn flaky, and I thought slightly more yellow than normal, but the milker made use of it in the ordinary way. Cow's temperature two degrees above normal ; she coughed, and obviously not a healthy cow."

On 19. 2. 10. Mr Villar reported that this cow was much better in every way.

She was killed on March 11, 1910, and the results of the examination will appear in a subsequent report.

Cow 36.

The veterinary reports on this cow are given above. Counts of the cells from all four quarters were made on 31. I. 10 with the following results:

R. H.	12,400,000 cells per c.c.
L. H.	1,942,000 „ „
L. F.	172,000 „ „
R. F.	660,000 „ „

No streptococci were apparently present and these milks were quite normal in appearance.

The chemical analyses of the milk on 1. I. 10 were as follows:

	Fat	T. Solids	N. F. S.	Lactose	Protein & Ash
R. H.	3.35	12.08	8.73	4.74	3.99
L. H.	3.50	11.74	8.24	4.15	4.09
L. F.	3.50	12.81	9.31	4.78	4.53
R. F.	3.15	11.91	8.76	4.82	3.94

All were quite normal in appearance and the milk of the quarter (R. H.) which shows the high cell count in this case does not show a depressed lactose figure, which curiously enough is found in the milk of the L. H. quarter which quarter is not in any way affected.

The milk was again examined on 20. I. 10 with the following results:

R. H.	336,000 cells per c.c.
L. H.	1,560,000 „ „
L. F.	68,000 „ „
R. F.	168,000 „ „

Mr Villar reported as follows:

“The swelling is more diffused, assuming a chronic character and is extending into the mammary tissue.”

This is most interesting as the lesion is becoming worse, and yet the cell count has fallen off considerably, and the *quarter giving the highest count is one not affected.*

The milk of each quarter was analysed on 26. I. 10 with the following results:

	T. Solids	Fat	N. F. S.	Lactose	Protein & Ash
R. H.	13.62	4.80	8.82	4.60	4.22
L. H.	12.18	3.75	8.43	4.23	4.20
L. F.	12.80	3.70	9.10	4.97	4.13
R. F.	12.34	3.70	8.64	—	—

All were quite normal in appearance. Here in the milk of the L. H. quarter, which now gives the highest cell count, the sugar is still depressed, but protein is normal.

Experimental Herd.

Six cows of the experimental herd of the Reading University College Farm at Shinfield were selected and samples received weekly from these cows as in the other cases.

These cows had calved as follows :

Ref. No. 19	March 19th, 1909.
„ „ 17	„ 1st, „
„ „ 16	Feb. 1st, 1909.
„ „ 11	Dec. 20th, 1908.
„ „ 9	Nov. 3rd, „
„ „ 14	May 24th, 1909.

The first sample of milk was received on June 30th, 1909.

The following are the results obtained :

Number of cows	Date	Cells per c.c.	Remarks
Six	30. 6. 09	67,000	—
„	7. 7. 09	88,000	—
„	14. 7. 09	126,000	—
„	28. 7. 09	70,000	—
„	4. 8. 09	67,000	—
„	11. 8. 09	200,000	—
„	18. 8. 09	184,000	—
„	25. 8. 09	304,000	—
„	31. 8. 09	161,000	—
„	7. 9. 09	212,000	—
„	15. 9. 09	830,000	Sudden heavy feed of green maize—no illness.
„	23. 9. 09	195,000	
„	29. 9. 09	189,000	—
„	6. 10. 09	359,000	—
„	13. 10. 09	165,000	—
„	19. 10. 09	228,000	—
Five	26. 10. 09	188,000	—
„	4. 11. 09	180,000	—
„	9. 11. 09	200,000	—
„	16. 11. 09	248,000	—
„	23. 11. 09	310,000	—
Three	30. 11. 09	126,000	—
„	7. 12. 09	137,000	—
„	14. 12. 09	139,000	—
Two	28. 12. 09	173,000	—
„	4. 1. 10	287,000	—
„	11. 1. 10	183,000	—
„	18. 1. 10	1,258,000	No disease.
„	25. 1. 10	1,015,000	

Except for the incident of the heavy feed with maize, the cell count of these cows pursued a remarkably steady course. No illness beyond indigestion appeared at any time, and even this latter had no effect on the cell count.

The milk of this herd is hardly comparable with ordinary dairy farming, but it is very interesting in that it shows that rest and regular habits may have a good deal to do with keeping a steady low cell count.

The two last cows which at the end showed an increased cell count were nearly dry and both were in calf; the increased count is therefore to be expected (cf. the last cows of Dairy Farm B, where the cows were barren).

General Summary of the foregoing Quantitative Examinations.

In the following table are given some of the counts obtained from mixed milks of cows either all healthy, or some healthy and some abnormal, in order that they may be easily compared:

Healthy Cows.		Healthy and Abnormal.	
No. of cows	Cells per c.c.	No. of cows	Cells per c.c.
6	726,000	5	483,000
5	817,000	6*	885,000
4	829,000	6*	1,073,000
5	1,070,000	5	1,075,000
6	1,186,000	6*	1,174,000
4	1,210,000	5*	1,295,000
5	1,450,000	6*	1,364,000
5	1,580,000	4*	3,290,000
5	1,678,000		
5	1,638,000		
4	1,735,000		
4	4,255,009		

* These contained the milk of two abnormal cows, the other samples only one.

The above figures show conclusively how little reliance can be placed on a count of the cellular elements as an indication of the presence of udder disease, even in such small numbers of cows as were here employed. Attention has already been directed to the fact that in cases of catarrhal mastitis, there is often no warning given in the way of increased cell count until the actual onset of the disease.

A comparative study of the cows, whose milk was examined separately on several occasions because a high cell count had occurred, leads to some interesting results:

Of the two cows which actually developed a typical catarrhal mastitis, viz. Cow 34 Farm D, and Cow 28 Farm B, it is to be noted

that in both cases, in the unattacked quarters, low cell counts were found on the occasions recorded, viz. soon after the onset. It is true that the cell count usually increases in the other quarters gradually, and particularly if they also succumb to the disease, but in neither of these cases was there any further obvious spread of the lesion. As we have already pointed out the cell count gives no indication of the state of affairs.

Cow 37 Farm D provides a very interesting study. There was undoubtedly considerable disturbance in the udder of the cow progressively involving nearly all four quarters, but particularly the two hind quarters.

This disturbance could not be described as acute or as suppurative mastitis. Chemical analyses constantly showed the milk to be but slightly altered, and then only in such a way as we have always found when the cell content is increased from any cause. The milk was certainly slightly slimy due undoubtedly to the large cell content, and on some occasions it was stated to be slightly discoloured and abnormal in odour, but this did not come under our own observation. The whole condition of the udder indeed suggests that there was a want of tone and lowered vitality of that organ, and that the abundance of streptococci might have been a sequel rather than a cause of condition. It must not be forgotten that she "ran her milk."

The milk of this cow though laden with streptococci was sold, and used locally. We had sufficient faith in our view of the case not to hinder the sale of this milk. The presence of her milk might have been detected in a mixed milk as the cell counts were often very high, but the diagnosis of any danger to the consumer is, in our opinion, doubtful.

This opinion is further supported by some recent experiments made by Savage (1910) for the Local Government Board in connection with his "goat-test" for the differentiation of streptococci. The experiments seem to show very conclusively that sore throat in human beings is not caused by the streptococci found in bovine mastitis.

Savage went so far as to test the truth of this conclusion by personal inoculations with such streptococci, from which no harm whatever resulted.

Of the effect of what may be taken as external agencies, Cow 36, Farm D, and Cow 6, Farm C, provide good instances. In the case of the former the stimulus took the form of a circumscribed superficial semi-acute inflammatory process, which during the course of our observation slowly spread. It is doubtful whether the actual secreting

tissue was involved, but an increased cell count followed. The same result was observed in the case of the other cow, but here the nature of the lesion is exceedingly doubtful. It was probably caused in the first instance by a slight blow, though no diminution of milk followed. When the cow was killed on 14. II. 10 all trace of the thickening had gone. A similar case is reported by Hastings and Hoffmann (1909, p. 469). The persistent effect of this hardening, even though it was rapidly disappearing in the case of our own cow, is remarkable.

The case of Cow 1, Farm A, also shows in a very clear manner the long persistence of cell proliferation after a slight and transitory udder trouble. In this case too the lesion was scarcely such as to give any reason to suppose that the milk would be unwholesome; yet, to the end of the lactation period the response to the early stimulus was maintained almost unimpaired. It must of course be remarked that cows which have already lost a quarter through mastitis are well known to be liable to a recurrence of the trouble, and it may be suggested that the activity produced in the germinal and epithelial layers of the udder by the first attack lessens their resistance to other attacks, perhaps on account of the continuity of the epithelial layer being constantly disturbed by the elimination and replacement of its units.

Such a lessened resistance and liability to recurrence is of course well known in connexion with various inflammatory lesions.

In Cow 2, Farm C, and Cow 27, Farm B, and possibly also Cow 26, of the same farm, we have instances of high cell counts, often prolonged without any reason that it was possible to discover. To say that the cell count presupposes the disease is simply a "circulus in probando." Cow "Dorine," mentioned by Hastings and Hoffmann (*loc. cit.*) is a similar case.

The general impression that is forced on the investigator into this question after prolonged study, is the hopelessness of arriving at any really satisfactory explanation of the phenomena such as we have detected. The udder is evidently an organ so open to stimuli of a most varied nature, and yet showing practically only one form of response to such stimuli, that the cause is not to be diagnosed from the effect produced.

The idea so often held, that the cow is a stolid unimpressionable animal is quite erroneous. She shows all the response to outside influences that the human subject does. Cows are extremely nervous and often show profound changes in their milk when the usual surroundings or methods are changed, or if they are harassed or excited

in any way. The effect may be transitory, but it is none the less real. The wonderful power which a cow can exercise over the udder, such as retention of the milk when a new milker or unusual method of milking is employed, is quite well known to any who have had to deal with cows.

We are of the opinion that the cytological examination of milk does not admit of any inference of the existence of a diseased condition of the cows supplying the milk. It may point to the desirability of veterinary inspection, but gives no "a priori" grounds for condemnation of the milk.

The effect of Tuberculin injections on Cell Count.

In two cases milk was received from cows which were tested with tuberculin. The samples were taken 24 hours before the injection and three days after the following results:

	Time	No. of cells
I.	24 hours previous	95,000
	3 days after	87,000
II.	24 hours previous	251,000
	3 days after	241,000

It is evident that injections of tuberculin do not cause an increase in the number of cells.

II. The nature of the cellular elements present in milk.

The question of the nature of the cells has been investigated in the course of the work done on the number of these elements present in the samples of milk examined. From the deposit obtained by centrifugalising as detailed below, stained films have been prepared by the following method, and a large number of preparations have been studied.

The method of preparation of the stained films is as follows:

5 c.c. of the milk (which has been exposed to the action of formalin (see above)) are diluted with 5 c.c. of physiological (0.8 %) salt solution, rotated sufficiently and all the supernatant liquid then removed. (The cream is entirely washed away as in the case of obtaining deposits for counting.) The deposit is diluted to 10 c.c. with distilled water shaken up and again rotated, the supernatant liquid removed and the deposit mixed up with sufficient distilled water by blowing through a fine glass pipette. 1 c.c. of water is usually sufficient dilution, but if the deposit be large a proportionately larger quantity of water is required. The diluted deposit is distributed over two perfectly clean cover slips set on a level table, a cover being suspended over them to prevent the ingress of dust; they are allowed to dry by

evaporation. They are then placed in alcohol-ether (1 : 1) for 30 minutes, allowed to dry and stained as under :

Stain. Modified Geimsa.

Azur II Eosin	3.0 grams
Azur II	1.6 „
Glycerin (Kahlbaum)	250 „
Methyl Alcohol (Kahlbaum)	250 „

The dry stains must be left in a desiccator for 48 hours before use. For use, place three to four drops of this stain in a large weighing bottle (2 inches high by $1\frac{1}{2}$ inches in diameter) and add 1 c.c. of water. Mix well and add 1 c.c. pure methyl alcohol. The films prepared as above are placed face upwards at the bottom (one to each bottle) and left for 48.—72 hours. At the end of this time 2 c.c. of a very dilute solution of acetic acid are added, consisting of acetic acid (Kahlbaum's absolute) 1.5 pts. per 100,000, mixing well. After four minutes pour off the stain and wash the film well for 30—40 secs. with distilled water by irrigation.

Dry and mount in Canada balsam. The time of action of the acetic acid may require modification in the case of individual observers. It must be allowed to act sufficiently long to cause the cytoplasm to stain pink and yet leave the nuclei a deep blue.

This method gives films in which the various cells on the whole are well stained and well differentiated. It is much superior to specimens prepared with methylene blue, borax methylene blue and eosin thionine blue, or with the Leishman stain. A few films have also been stained with haematoxylin and eosin after fixing: this also gives satisfactory specimens. From a study of a large number of films prepared in this way cells having the following characters may be distinguished.

(1) Cells having a large single nucleus (*large uni-nucleated cells*)¹.

These are roundish cells 9—12 μ in diameter, each containing a single nucleus, which occupies about half the cell. The nucleus is roundish, or sometimes elongated or semi-lunar in form, generally excentric, often quite on one side of the cell and then frequently semi-lunar in shape. The border of the nucleus is slightly ragged, the nucleoplasm stains deeply of a haematoxylin-blue colour, but not uniformly, showing lighter and darker irregularly-shaped portions.

The cytoplasm is structureless and stains well with the eosin. (In a few of these cells a double adjacent nucleus is present as though division of the nucleus had just occurred.) In many specimens this type is the predominant cell present.

Frequently naked nuclei, resembling the nucleus above described, are present. Cells apparently of this type are also met with, but in which the cytoplasm and nucleus are vacuolated and striated, so that no clear picture of the cell can be obtained. These are probably degenerate forms, or may have contained fat-droplets.

This large uni-nucleated cell is regarded as an epithelial cell derived from the secreting layer of the gland tissue. In the figures attached to Winkler's paper (*loc. cit.*) cells with semi-lunar marginal nuclei are here and there depicted. Winkler also mentions the occurrence of naked nuclei (VIII).

¹ The prefixes 'uni' and 'multi' have been adopted in describing the cells, to avoid any possibility of confusing them with the 'mono-' and 'poly-' nuclears of the blood.

(2) Cells having two or more small nuclei (*multi-nucleated cells*)¹.

These are roundish cells, smaller than the preceding, being about 8—10 μ in diameter. Each has 2—4, occasionally 5, small, generally roundish, occasionally irregular, nuclei, staining deeply and uniformly of a deep haematoxylin-blue colour. The nuclei may be separate, scattered or clustered, and are sometimes arranged in a crescent or horse-shoe. The cytoplasm is structureless and stains well with the eosin.

Similar cells are also present, but the nuclei of which stain a pale Cambridge blue. The two kinds are probably identical, the latter being perhaps degenerated.

If the nuclei are arranged crescentrically, or in a horse-shoe, these cells bear some resemblance to polymorphonuclear leucocytes, particularly if badly stained. When properly stained however, there can be no question that they are *not* polymorphonuclear leucocytes.

These cells are sometimes almost absent, sometimes they are numerous, sometimes abundant.

There can be little doubt that these are the "germinal cells" described by Winkler.

(3) Cells with a small single nucleus (*small uni-nucleated cells*).

These are roundish cells 7—9 μ in diameter. The nucleus is small with sharp edges, roundish, and stains uniformly and deeply of a haematoxylin-blue colour. The cytoplasm is structureless and stains well with the eosin, sometimes deeply, in which case the cell resembles a normoblast ('normoblastic' type). In some of these cells which stain deeply with eosin, the nucleus stains a Cambridge blue. These cells are generally scanty in numbers.

(4) Cells with eosinophilic granules (*eosinophile cells*).

These are roundish cells 7—9 μ in diameter. The nucleus is lobed or horse-shoe shaped and stains a Cambridge blue. The cytoplasm is filled with fine eosinophilic granules.

These cells are always scanty in numbers, or frequently absent.

(5) Vacuolated cells.

Cells of some size (10—15 μ) without, or with a faint staining, large, single nucleus. The cytoplasm stains feebly and appears to be vacuolated. These cells are probably fat-bearing cells, cells which have undergone fatty degeneration, or cells allied to colostrum corpuscles.

They are generally present in small numbers in all specimens.

(6) Cells of indeterminate nature.

A certain proportion of cells is generally present in all specimens which are indefinite in character, owing to feeble staining of cytoplasm and nucleus, giving them a hazy appearance, and cannot be classified under the above headings. An occasional lymphocyte-like cell is seen, also squamous cells.

¹ The prefixes 'uni' and 'multi' have been adopted in describing the cells, to avoid any possibility of confusing them with the 'mono-' and 'poly-' nuclears of the blood.

Cells also occur which cannot be definitely classified under the above heads and may have characters intermediate between the cells of one and another class. It would serve no useful purpose, though it might be done, to make groups to include these cells, and in the descriptions of the specimens which follow, a general survey of each film has been made and the general nature of the cells present is summarised.

The outstanding feature in the examination of some hundred films, prepared both from normal cows at different periods of lactation and from cows presenting slight signs of mastitis and representing many thousands of cells, is that *no cell having any decided resemblance to a polymorphonuclear leucocyte has been detected*, and phagocytosis of bacteria present is conspicuous by its absence.

We are not prepared at the present time to refer the cells other than those included under the headings 1 and 2 to any particular tissues of the udder until our investigation on the histology of the udder is completed, when reference will be again made to them.

*General description of cells found on microscopical examination
of stained films of milk deposits.*

Mixed milk of six healthy cows.

FARM B.

No.	Date	Cells present
I.	7. 4. 09	Mostly of the large uni-nuclear type with rounded nuclei; some with divided nucleus. An occasional multi-nuclear and small uni-nuclear.
II.	14. 4. 09	Mostly of the large uni-nuclear type. The staining in this specimen is somewhat hazy and indefinite.
III.	21. 4. 09	Almost entirely of the large uni-nuclear type, mostly with rounded, a few with semi-lunar or horse-shoe nucleus. A few small uni-nuclears of the normoblastic type also present.
IV.	28. 4. 09	Almost entirely of the large uni-nuclear type, mostly with rounded, a few with horse-shoe, nuclei.
V.	5. 5. 09	Almost entirely of the large uni-nuclear type with rounded nuclei.
VI.	11. 5. 09	Mostly of the large uni-nuclear type with rounded nuclei. A few small uni-nuclears.
VII.	8. 6. 09	Mixture of cells of large uni-nuclear with some of the multi-nuclear types. (Haematoxylin and eosin staining.)
VIII.	15. 6. 09	Mostly of the large uni-nuclear type, both with rounded and with semi-lunar nuclei. Some small uni-nuclear cells.
IX.	24. 6. 09	Same as VIII.
X.	7. 7. 09	Majority of cells are large uni-nuclears with some multi-nuclears and a few small uni-nuclears.

EXPERIMENTAL HERD.

No.	Date	Cells present
XI.	7. 7. 09	Mostly multi-nuclears with relatively numerous small uni-nuclears. Some vacuolated cells. Hardly any large uni-nuclears.
XII.	18. 8. 09	Much the same as XI.

FARM C.

XIII.	28. 4. 09	Mostly large uni-nuclear cells with rounded nuclei, some with divided nuclei, with a few multi-nuclear cells.
XIV.	11. 5. 09	Same as XIII.
XV.	8. 6. 09	Large uni-nuclears with some multi-nuclears and a few small uni-nuclears.
XVI.	24. 6. 09	Mostly large uni-nuclears with a few multi-nuclears, an occasional small uni-nuclear and here and there an eosinophile.

Milk of newly calved cows.

FARM D.

No.	Date	Nature of slide	Cells present
I.	26. 10. 09	Cow 33 (Cow remained healthy)	Almost entirely of large uni-nuclear type with rounded nuclei. Some vacuolated cells.
II.	3. 11. 09	Cow 34 (Developed severe mastitis about 24. 11. 09)	Large uni-nuclear cells with some multi-nuclear and vacuolated cells. Large number of red-blood corpuscles.
IIa.	4. 11. 09	Cows 33 and 34 Mixed milk	Considerable number of multi-nuclear cells with some large uni-nuclear and vacuolated cells.
III.	10. 11. 09	Cow 35 (Remained healthy)	Mixture of large uni-nuclear, multi-nuclear and vacuolated cells (not well stained).
IV.	17. 11. 09	Cow 36 Developed a slow external inflammation of the udder R. H. quarter	Mostly multi-nuclear cells with a few large uni-nuclear, small uni-nuclear and vacuolated cells.
V.	24. 11. 09	Cow 38 (Remained healthy)	Large uni-nuclear and vacuolated cells with a few multi-nuclears.

Milk of single cows.

Cow 1. FARM A.

{	I.	5. 5. 09	Mixed milk of all quarters	A mixture of large uni-nuclears with multi-nuclears. Small uni-nuclear cells very scanty. A few vacuolated cells. No eosinophiles. Many of the large uni-nuclears have a semi-lunar nucleus.
	II.	5. 5. 09	Milk of Cow I. as above mixed with milk of 5 healthy cows.	Much the same as I. but multi-nuclear cells scanty. Many of the large uni-nuclears show an apparently dividing or divided nucleus.

No.	Date	Nature of slide	Cells present
III.	12. 5. 09	Mixed milk of all 3 quarters.	Much the same as II.
IV.	12. 5. 09	Milk of Cow I. and the milk of 5 healthy cows.	Much as II.
V.	19. 5. 09	Mixed milk of two sound quarters	Mostly of the large uni-nuclear type: not so well stained as usual. Many show the semi-lunar or divided nucleus. Hardly any other type of cell present; practically no multi-nuclears.
VI.	19. 5. 09	Milk of affected quarter	Much the same as I. but a few small uni-nuclears and vacuolated cells present in addition.
VII.	26. 5. 09	Two sound quarters	Practically all of the large uni-nuclear type.
VIII.	26. 5. 09	Affected quarter	Much the same as VII. Cells nearly all of the large uni-nuclear type, with a few small uni-nuclears mostly of the normo-blastic type.
IX.	1. 6. 09	Two sound quarters	Preponderating cells are of the multi-nuclear and small uni-nuclear types with a small admixture of large uni-nuclears.
X.	1. 6. 09	Affected quarter	Apparently mostly of the large uni-nuclear type, but the nuclear staining very poor and the cells therefore indefinite.
XI.	8. 6. 09	Sound quarters	Admixture of cells of the large uni-nuclear and multi-nuclear types.
XII.	8. 6. 09	Affected quarter	The same as the sound quarters with some small uni-nuclear cells.
XIII.	11. 6. 09	Sound quarters	Almost entirely of the large uni-nuclear type.
XIV.	15. 6. 09	Affected quarter	An admixture of large uni-nuclears and multi-nuclears with a few small uni-nuclears.
XV.	26. 6. 09	Sound quarters	Mostly of the large uni-nuclear type with semi-lunar nuclei.
The milk was again examined two months later.			
XVI.	30. 8. 09	Affected quarter	Mostly of the large uni-nuclear type with semi-lunar nuclei together with a few multi-nuclear cells, and an occasional small uni-nuclear.
XVII.	30. 8. 09	Sound quarters	Same as last with in addition a few vacuolated cells.

Cellular Elements in Milk

Cow 26. FARM B.

No.	Date	Nature of slide	Cells present
I.	7. 8. 09	Mixed milk. High cell count. Diminished milk. No disease developed	Mostly multi-nuclears with some vacuolated cells. Large and small uni-nuclears scanty.
II.	18. 8. 09	Milk of two quarters with low count	Some multi-nuclears, relatively large number of small uni-nuclears, and some vacuolated cells. Large uni-nuclears almost absent. A few red-blood corpuscles. (Staining indifferent.)
III.	18. 8. 09	L. F. quarter High count	Mostly multi-nuclears. Relatively large number of vacuolated cells. A few small uni-nuclears. Large uni-nuclears almost absent. No red-blood corpuscles.
IV.	7. 9. 09	L. F. quarter	Mostly multi-nuclears. A few large and small uni-nuclears. Relatively large number of vacuolated cells. No red-blood corpuscles.

Cow 27. FARM B.

I.	7. 8. 09	Mixed milk. High count. No disease developed	Mostly multi-nuclears with a few large uni-nuclears and vacuolated cells.
II.	8. 11. 09 (Cow nearly dry)	L. H. quarter	Much the same as I.
III.	8. 11. 09	L. F. quarter	Ditto, but preponderance of multi-nuclears.
IV.	8. 11. 09	R. F. quarter R. H. (No milk)	Ditto, with relatively numerous eosinophiles.
V.	10. 1. 10 (After calving)	Mixed milk High count	Some large uni-nuclear and vacuolated cells, many multi-nuclears and small uni-nuclears.

Cow 2. FARM C.

I.	10. 12. 09	L. H. quarter, very high count (21,000,000) from this quarter	Multi-nuclears with a smaller number of large uni-nuclears. Some vacuolated and eosinophile cells.
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Cow 6. FARM C.

I.	18. 8. 09	R. H. quarter, high count. No apparent disease	Mostly multi-nuclears with some large and small uni-nuclears, many of the latter of the normoblastic type.
II.	18. 8. 09	Two other quarters Low counts	Number of small uni-nuclears. Some multi-nuclears. Large uni-nuclears scanty (staining indifferent).

No.	Date	Nature of slide	Cells present
III.	15. 1. 10	R. H. quarter, high	Multi-nuclears with a smaller number of large uni-nuclears. Some small uni-nuclear and vacuolated cells.
	(Nearly dry)	count from this quarter	
IV.	15. 1. 10	L. H. quarter	Same as R. H. quarter.
Cow 37. FARM D.			
I.	8. 12. 09	Mixed milk	Considerable number of multi-nuclear cells with some large and small uni-nuclear and vacuolated cells.
II.	13. 12. 09	R. H. quarter, giving heavy deposit	Large uni-nuclears with some multi-nuclears and a few small uni-nuclears. Some streptococci.
III.	2. 1. 10	R. H. normal in appearance but heavy deposit	Same as I. with some vacuolated cells. Many of the large uni-nuclears with semi-lunar and horse-shoe nuclei. Many long chain streptococci.
	2. 1. 10	R. F. normal	
	2. 1. 10	L. F. normal	
	2. 1. 10	L. H. swollen, heavy deposit	
VI.	2. 1. 10	L. H. swollen, heavy deposit	Many multi-nuclears with large and small uni-nuclears and some vacuolated cells. Long chain streptococci abundant.
VII.	3. 2. 10	Milk of two fore-quarters mixed. Normal in appearance	Large uni-nuclears and multi-nuclears. Some of the large uni-nuclears with semi-lunar nuclei and vacuolated. A few small uni-nuclear and vacuolated cells. Long chain streptococci abundant.

Cow A.

This cow was not one used in the preceding investigations, but was a healthy cow which received a blow, probably a kick, near the base of the R. H. quarter. The milk of the quarter was much diminished in quantity and streptococci (pathogenic to rabbits) appeared in this quarter. There was no outward sign of disease beyond the bruise, and on slaughtering the animal later, only a hard mass in the neighbourhood of the bruise was found, there being a very slight area of inflammation round this. Very heavy deposits were given by the milk from the bruised quarter.

I.	21. 4. 09 (Immediately after accident)	Mixed milk	Mostly large uni-nuclears, many of which show divided nucleus. Some multi-nuclears and a few small uni-nuclears. Some masses of long chain streptococci.
II.	28. 4. 09	Mixed milk of three good quarters	Mostly large uni-nuclears, some with semi-lunar or horse-shoe nucleus. Multi-nuclears comparatively scanty. A few red-blood corpuscles.

No.	Date	Nature of slide	Cells present
III.	5. 5. 09	Bruised quarter	Mostly large uni-nuclears, some with semi-lunar or horse-shoe nucleus. A few multi-nuclears.
IV.	12. 5. 09	Bruised quarter	Apparently mostly large uni-nuclears, which however are not well stained. Abundant long chain streptococci.
V.	12. 5. 09	Three sound quarters	Mostly large uni-nuclears with some multi-nuclears. A few small uni-nuclears and vacuolated cells also present.
VI.	19. 5. 09	Bruised quarter	Almost entirely large uni-nuclears, which however are not well stained.
VII.	19. 5. 09	Three sound quarters	Almost entirely large uni-nuclears.

SPECIAL EXAMINATIONS.

I.	3. 2. 10	Milk of two cows (healthy and in calf) but nearly dry. High count	Large numbers of multi-nuclear cells present, both those with deep- and those with pale-staining nuclei. The large uni-nuclear cells are less numerous.
II.	3. 11. 09	Milk of three cows, nearly dry. (Barren)	Large uni-nuclears with a good many vacuolated cells and a few multi-nuclears.
III.	19. 1. 10	Milk of four cows, nearly dry. (Some in calf)	Mixture of all kinds of cells—large and small uni-nuclears, multi-nuclears, vacuolated and few eosinophiles.
IV.	17. 5. 09	Milk of cow before tuberculin injection	Abundance of large uni-nuclear and multi-nuclear cells. Fair number of small uni-nuclear (ordinary and normoblastic type) and vacuolated cells. No eosinophiles.
IV a.		Milk of cow after injection. (Cow reacted but tuberculosis slight)	Practically the same as before injection.
V.	10. 6. 09	Milk from cow suffering from cowpox	Mostly multi-nuclears with a few large uni-nuclears and an occasional small uni-nuclear.
VI.	24. 8. 09	Cow 28, Farm B R. H. moderate count	Mostly large uni-nuclears with a few multi-nuclear and vacuolated cells.
VII.	24. 8. 09	R. F. and L. F. mixed milk. Low counts	Much the same as R. H. quarter.
VIII.	24. 8. 09	Mastitis deposit from serous liquid	Almost entirely large uni-nuclears, many with semi-lunar or horse-shoe nuclei.
IX.		Mastitis deposit from serous liquid. Cow 34	Almost entirely multi-nuclear cells, with an occasional large uni-nuclear and vacuolated cell. No streptococci. Nothing like a polymorphonuclear leucocyte present.

CONCLUSIONS.

It is difficult to formulate any general conclusions from this survey of the kinds of cells present in different conditions. All that can be said is, that in the milk of healthy cows in full milk and which do not give a high cell count, the majority of cells tend to be of the type termed "large uni-nuclears," with a small admixture of other cells. At the beginning and end of lactation, or when the cell count is high, the multi-nuclears tend to be the predominant cell, and this is the case whether the high cell count is without discernible cause, or whether a definite mastitis is present. That is to say, a high cell count seems to be due to an increase of the multi-nuclears, and may or may not be associated with mastitis. These conclusions are in accordance with the hypotheses we have put forward as to the effect of various stimuli on the gland tissue of the udder. Substituting the word "polymorphonuclear leucocyte" for "multi-nuclear cell," our results are in general in accord with Savage (1908, p. 33), *but we differ entirely as to the nature and origin of the actual cellular elements*. Even in the deposits from the serous fluid in catarrhal mastitis we do not find the presence of polymorphonuclear leucocytes, and must conclude that the cells of the deposit are not "pus cells" in the ordinary acceptation. *It is not in our opinion possible to recognise diseased conditions by means of a microscopical examination of the cells present*.

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THE CONTAMINATION OF ICE-CREAM.
A SANITARY AND BACTERIOLOGICAL STUDY.

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Plate VII.

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THE importance of ice-cream as a means by which infection may be carried and disease produced has not entirely escaped the attention of epidemiologists. In some recorded cases a particular ingredient of the ice-cream has been at fault, while in others it has been the ice-cream as a whole which has become contaminated.

Recorded outbreaks due to ice-cream.

Scarlet Fever. Buchanan (1875) called attention to the fact that ice-pudding had caused cases of this disease. He traced the infection to the cream which had been used in the manufacture of the implicated food-stuff.

Typhoid fever. Turner (1892) traced the origin of an outbreak of typhoid fever occurring at Deptford to specifically infected ice-cream made by Italians living in Mill Lane. His investigation during this outbreak into the conditions under which ice-cream is manufactured led him to conclude that the sale of ice-cream should be regulated in the same way as the sale of milk.

Munro (1894) also traced an epidemic of typhoid fever in Renfrewshire to infected ice-cream.

Hope (1897) records 25 cases of typhoid fever as resulting from the consumption of ice-cream bought at a village fair. A case of typhoid fever was resident in the house of the vendor at the time.

Nineteen cases of typhoid fever are recorded by Barras (1904) as having been due to ice-cream sold by a vendor who was suffering from typhoid fever, which he considered to be influenza.

Diarrhoea. Henry (1900) describes an epidemic of this disease involving 146 persons with 1 death which he attributed to ice-cream. The baby in the house of the vendor was suffering from diarrhoea, and Henry reports that its napkins were washed within a few inches of the strainer used in the manufacture of ice-cream.

"Gaertner" Infection. A record of an outbreak of disease due to the consumption of ice-cream is contributed by Robertson (1905). 52 cases were recorded, 4 being adults and 48 children under 14 years of age. All had partaken of ice-cream supplied by one vendor, and a sample of this was submitted for bacteriological analysis. The examination revealed the presence of a bacillus belonging to the Gaertner group, and to this organism the outbreak was consequently attributed.

Peacock (1909) records an outbreak of ice-cream poisoning in Attleborough, in which 67 persons were affected between the ages of 18 months and 50 years. The *Bacillus enteritidis* of Gaertner was found in a sample of faeces obtained from one of the sufferers, while the blood of another agglutinated a laboratory culture of this bacillus.

Ice-cream poisoning. Collingridge (1902) attributed the illness of 18 boys in the telegraph department in the City of London to the

consumption of ice-cream. The main symptoms of the illness were epigastric pain, colic, headache, nausea and nervous depression associated in some instances with vomiting and diarrhoea.

Previous bacteriological studies of ice-cream.

The foregoing accounts show that ice-cream may be the carrier of many diseases. The first systematic study of the frozen commodity from the bacteriological point of view was conducted in this country by MacFadyen and Colwell (1895). Their investigations included chemical, microscopic and bacteriological examinations. Bed bugs, bugs' legs, fleas, straw, human hair, cats' and dogs' hairs, coal dust, woollen and linen fibres, tobacco, epithelial scales and muscular tissue were all revealed as occasionally polluting this material. The maximum number of organisms per cubic centimetre in ice-cream which these observers found present in shop samples was just over 1,000,000 and in barrow samples over 7,000,000.

Neild-Cook (1896) gave attention to the subject in the following year. He obtained 14,280,000 microbes per cubic centimetre in ice-cream, and was able to isolate from several samples the *Bacillus coli communis*, *Proteus vulgaris*, *Bacillus fluorescens liquefaciens* and many cocci.

Wilkinson (1899) has also given attention to the bacteriology of ice-creams.

Klein (1902) gave the results of the bacteriological examination of twenty-four samples of ice-cream collected in the City of London. He reported that the number of organisms per cubic centimetre varied greatly, and that thirteen of the samples were proved poisonous by inoculation into guinea-pigs. Many organisms of the coli group were isolated during the investigation and one of them was an extremely virulent bacillus. The majority of the organisms were non-sporing and Klein was therefore of opinion that contamination occurred after boiling, *i.e.* during the cooling and freezing processes.

Rickards (1906) furnishes records of the bacteriological examinations of various samples of ice-cream and hokey-pokey in which the highest bacterial count was 150,000,000 per cubic centimetre, and the lowest 1,000,000. Reference must also be made to an account of similar work conducted in the bacteriological laboratory of the City of Philadelphia by Pennington and Walter (1907). 68 samples were examined, and among these the lowest count recorded was 50,000 per cubic centimetre, and

the highest more than 151,200,000 and called innumerable. Their examination included an enumeration of leucocytes and a search for streptococci as well as a sanitary inspection of the premises on which the article was manufactured.

Scope of the present investigation.

The present research has been undertaken in Birmingham with a view of ascertaining how and to what extent ice-cream is contaminated. It has been limited to the ice-cream prepared by Italians and small retail English confectioners and has included

- (1) An enquiry into the methods of preparation,
- (2) A sanitary inspection of the premises on which ice-cream is made, and
- (3) A bacteriological study of the mixture at various stages in the process of manufacture.

Composition of ice-cream in this country.

The ingredients vary according to the quality of the article sold. The simplest form of ice-cream is composed of milk, sugar and cornflour. In others the cornflour is replaced by "ice-cream powder," and to either combination eggs may be added in the proportion of 4 to 12 to the gallon. Sometimes a colouring agent is added and the finished product varies in colour from white to orange.

Methods of preparation.

The methods of preparation by 50 different vendors (33 English and 17 Italian) were investigated.

Heating. The first stage of the process varies according to the practice of the manufacturer. In four instances (all English) the milk and sugar were boiled in an enamel pot and poured with stirring on to the remaining ingredient or ingredients in a galvanised iron bucket.

In 17 instances (4 Italian and 13 English) some form of "water-bath" was used for heating all the ingredients together. In this method the ice-cream ingredients are put into a freezer, which is then placed in water and gradually heated. In only one of the seventeen instances was a boiler reserved for use as a water-bath, the other 16 manufacturers using washhouse boilers or large iron pots which were used also for other purposes.

In the remaining 29 instances (16 English and 13 Italian) the milk and sugar were boiled in enamel or iron pots directly over an open fire-place or stove and the cornflour or ice-cream powder and eggs, if any, were added gradually with stirring and maintenance of the temperature.

The duration of heating varied greatly. In the first method the milk and sugar were merely brought to the boil, while in the second and third methods the manufacturers stated that heating was continued in some cases for a few minutes and in others for an hour and a half.

Cooling. After heating the ice-cream commodity is set out in galvanised iron buckets to cool, or is allowed to remain in the freezer if the first stage was carried out in that vessel. In only one instance were these buckets reserved solely for use in the manufacture of ice-cream. In the remaining 49 cases they were used for ordinary domestic purposes as well, and in one instance for tripe cleansing also. The vessels in which the ice-cream was cooled were usually placed on a slope so as to expose a large surface to the air for cooling, and were allowed to stand in this position over night.

In 10 cases (8 Italian and 2 English) the ice-cream was covered while cooling, and with regard to the 8 Italians it may be said that in no instance was the covering efficient. It consisted of curtain material with a wide mesh work which could prevent only large particles of dirt gaining access. The two English vendors used fine muslin. No special attention was given to the washing or cleansing of these coverings which were used over and over again until obviously unclean. In several cases the covers dipped into the ice-cream while cooling.

Freezing. On the following morning the mixture was strained into the freezer (in those cases in which it had not cooled in the freezer) through a metal sieve. The freezers used were either of the English or the American pattern. Salt and ice formed the freezing mixture employed and were usually specially supplied by a trader for the purpose, but in a few instances salt was used which had been previously employed in the curing of bacon. Lumps of ice are frequently put into the ice-cream mixture to hasten freezing. As no precautions are taken to secure that the ice is clean, its addition in this way greatly adds to the contamination of the frozen product.

The English freezer. Where an English freezer is used the ice-cream has to be stirred up frequently to hasten and complete its solidification. For this purpose a special metal spade with a wooden

handle¹ is employed, and the lid of the vessel is removed and usually not replaced. The spade is worked by the hands which have not been washed before freezing is begun and which, sliding up and down the handle of the spade and being used occasionally for tasting the ice-cream, greatly add to the contamination of the finished product.

The American freezer. When an American freezer is used the lid is kept on constantly during the freezing process, and the necessary stirring is done by a mechanical contrivance inside the apparatus. This freezer obviously greatly diminishes the risks of pollution, and it ought to be in general use.

The premises and their surroundings.

Twelve manufacturers (all Italians) had sheds erected for the preparation of the article. Only one of these twelve had a place used solely by himself. The others used sheds in common with other vendors. These sheds were situate in a common yard and were usually constructed of wood carelessly nailed together, with a corrugated iron roof on which were to be found old baskets, rabbit skins, and other rubbish. The floors were badly paved. In several cases tubs overflowing with vegetable refuse were close at hand, and the w.c.'s in the common yard in which the manufacture of ice-cream was going on were frequently found in a filthy state, as a result of improper use by the Italian population.

Amongst the remaining thirty-eight vendors the process of heating was carried on in the living room, kitchen or scullery of the house², or in the washhouse common to several families, the ice-cream mixture afterwards being put on the scullery sink or the doorstep to cool. Freezing was done in the common yard or at the door in the street.

The storage of ice-cream.

Where no sheds had been built for the purpose of the trade in ice-cream, as in the case of the 12 manufacturers already alluded to, the frozen product was stored, usually uncovered, in a dirty underground

¹ This spade should be made in one piece (all metal) so that it can be thoroughly and easily cleansed.

² In the case of Italians the house is frequently overcrowded, *e.g.*, in one case three small bedrooms and two small living rooms were occupied by seven adults (over 15 years) and six children (under 14 years).

cellar, to which the dust from the street or yard had easy access through a perforated iron grating or grid. This cellar was also used for the storage of other materials, *e.g.* wood, coal, etc.

The cleansing of vessels.

All the manufacturers stated that the vessels employed in the preparation and sale were washed out with hot soda water, scalded, and thoroughly rinsed after use. It is doubtful if this process is always carried out, as in the majority of cases boiling water could be obtained in sufficient quantity only by heating over the kitchen fire in the same pot as was used for the preparation of the ice-cream. With regard to the glass vessels in which ice-cream is sold to the purchaser, it is to be noted that in the case of street trolleys the vendor has only a very small and limited supply of water with which to cleanse them after use.

The storage of vessels.

For the most part ice-cream is prepared on a Friday for sale during the week-end, and for the rest of the week the utensils used are stored anywhere as convenient, no special place being provided for them.

Sanitary classification of premises.

All the foregoing points were taken into consideration in classifying the various premises inspected, into clean, fair, dirty and filthy.

The following classification gives the results:

- 5 or 10 % were considered clean.
- 18 or 36 % were considered fair.
- 23 or 46 % were considered dirty.
- 4 or 8 % were considered filthy.

Collection of the samples.

The bacteriological examination was begun on the 7th of July, 1908. Three series of samples were taken called respectively, "a," "b," and "c."

"a" *Samples*: taken from each of 50 manufacturers immediately after the ice-cream material had been boiled. These samples were

taken directly out of the vessels in which the ingredients were heated.

"b" *Samples*: taken after the commodity had been cooled.

"c" *Samples*: taken after the material had been frozen.

All these samples were taken with sterile precautions in wide mouthed bottles of about 200 cubic centimetres capacity, and conveyed to the laboratory in a specially constructed case without delay. The frozen ice-cream never reached the laboratory in a melted condition.

Dilution of the samples for bacteriological examination.

It was always possible to deal with the samples taken immediately after boiling without dilution, as the preliminary quantities were put into the various media while the ice-cream material was still hot and before it had set. It was also practicable to deal in a similar way with several of the other samples. But with many of the cooled and all the frozen samples definite dilutions (usually half and half) with sterile distilled water had to be made.

The bacteriological examination.

The routine examination of these samples was as follows:

Test 1. An enumeration of the colonies capable of growing on nutrient gelatine (reaction + 1 %) at 20°—22° C. in 72 hours.

Test 2. A similar estimation using nutrient agar (reaction + 1 %) and incubating at 30°—37° C. for 48 hours.

Test 3. Deci-multiple quantities were put into Bile-Salt Glucose Broth, and after incubation at 35°—37° C. for 48 hours the reaction produced was noted. In those cases in which acid and gas were produced, a looplet was plated after appropriate dilution on Bile-Salt Lactose Agar and an endeavour made to determine the identity of the colonies by 16 different tests.

Test 4. An estimation of the number of spores of the *Bacillus enteritidis sporogenes* present.

Test 5. An estimation of the number of streptococci present by incubating deci-multiple quantities of the ice-cream in neutral Red Glucose Broth at 35° to 37° C. and examining microscopically the sediment. The character of the chains of streptococci, when present, were noted. Where these organisms as seen by the microscope appeared to be present in large numbers, attempts were made to isolate the microbe sometimes on Drigalski's medium and sometimes on Glucose Agar.

The following table shews the various quantities used for examination in the case of each series of samples :

TABLE I.

Shewing quantities examined for the five primary tests.

	" a "	" b "	" c "
Tests	Samples taken immediately after heating	Samples taken after cooling	Samples taken after freezing
1 and 2	·01 and ·0001 c.c.	·0001 and ·000001 c.c.	Same as " b ".
3 and 5	10, 1, ·1 and ·01 c.c.	·10, 1, ·1, ·01, ·001 and ·0001 c.c.	Same as " b ".
4	100, 10 and 1 c.c.	100, 10 and 1 c.c.	Same as " b ".

Test 1. The number of colonies per cubic centimetre capable of growing on nutrient gelatine (reaction + 1 %) at 20° to 22° C. in 3 days.

" a " samples. With regard to the samples taken immediately after heating it may be said that in 14 cases total liquefaction occurred within the three days' incubation. 1 was liquefied on the 1st day, 7 on the 2nd day, and 6 on the 3rd day. These samples are excluded therefore from the averages. In three instances no growth occurred with 0·01 cubic centimetre, and in these cases the results have been entered as less than 100 colonies per cubic centimetre. For the purposes of calculation they have been assumed to contain 100 colonies per cubic centimetre, and these were the lowest results recorded. In three cases the results were high, viz. 846,000 colonies, 10,000,000 colonies and 20,000,000 colonies per cubic centimetre respectively. Including all these results for the 36 samples which did not liquefy within three days, the average count was found to be 867,319 colonies per cubic centimetre. Excluding the three high counts already referred to, and taking the average of the remaining samples, the count becomes 11,439.

The preliminary heating is obviously therefore not a process which is conducted so as to secure a sterile product although sterilization of the ice-cream mixture can be accomplished at the first stage. The three samples which shewed less than 100 colonies per cubic centimetre may have been sterile. One of these was heated for an hour and a half in a water-bath amidst most filthy surroundings ; another was heated directly over the fire under quite the best conditions discovered during the inspections, while the third was heated in the same manner as the first under fair sanitary conditions. It is clear therefore that the sterility of the product at this stage depends on the heating alone.

The following laboratory experiments were conducted to ascertain how long it was necessary to heat the mixture of milk, sugar and corn-flour by the methods in vogue before it became sterile.

Experiment 1. Heating by "water-bath" method. Ice-cream mixture was heated in a double saucepan, the inner vessel containing the milk, sugar and cornflour being placed in the outer containing cold water which was gradually brought to the boil. A drop of the ice-cream mixture was put into nutrient broth with sterile precautions every 5 minutes for 40 minutes after the water had begun to boil. No growth resulted after 25 minutes.

The maximum temperature attained by the ice-cream mixture heated under these conditions was 92° C. It rose to this temperature after the water had boiled 10 minutes, and remained constant throughout the remainder of the experiment.

Experiment 2. Heating directly over flame. A similar experiment was conducted in which the ice-cream ingredients were boiled directly over the flame with constant stirring to prevent burning of the product. Drops of the mixture were put into nutrient broth at intervals of 2 minutes after boiling had begun and no growth was obtained after 8 minutes.

It is therefore fair to conclude that with the possible exception of the three samples shewing less than 100 microbes per cubic centimetre none had been heated at a sufficient temperature for a sufficiently long period to secure sterility, as heating by means of the water-bath for 30 minutes or directly over the flame for 10 minutes positively ensures a sterile article.

"b" samples. The second series of samples were taken at varying intervals after cooling had begun. One sample was taken only 1½ hours after it had been put out to cool, while the maximum period of cooling before taking the second sample was, in two instances, 28 hours. The average number of hours which these samples had been cooling was 15½. Seven gelatine plates of the "b" samples were liquefied completely in two days, and 8 in 3 days, *i.e.* 15 in all, and these plates are excluded from further consideration. The lowest count recorded was 20,000 organisms per cubic centimetre in each of two cases, and the highest 102,240,000. The average for all the 35 enumerated samples was 13,042,857, an enormous increase over the average for the samples taken immediately after heating, *viz.* 867,319. This is what is to be expected when the material in question is a nutrient fluid such as ice-cream exposed to contaminating conditions, and shews how important it is to have rapid cooling under clean conditions.

“c” samples. These samples were all taken after the material had been frozen. In one instance freezing was done after the sample had cooled $3\frac{1}{2}$ hours, but in two instances cooling had gone on for 44 hours before freezing was begun. The average time which elapsed between heating and freezing was about $20\frac{3}{4}$ hours for all the 50 samples examined. The “c” samples were taken on an average $2\frac{1}{2}$ hours after freezing. 38 of these samples were purchased when the article was exposed for sale in the street in the case of the Italian hawkers or in a shop in the case of the small confectioners. The remaining 12 samples were obtained immediately freezing was completed and before the finished product was placed on the market. 12 samples liquefied during the three days’ incubation and these are excluded from further consideration. The lowest count recorded was 50,000, the highest 3,800,000,000, and the average for the 38 enumerated samples 372,213,421 colonies per cubic centimetre. This average for frozen samples is higher than that obtained by previous investigators who, however, examined only a few samples. It is mainly accounted for by the samples yielding more than 1,000,000,000 organisms per cubic centimetre (five in number), excluding which gives an average of 16,470,515 organisms per cubic centimetre—a number in accord with other workers.

Test 2. Number of organisms capable of growing on nutrient agar (reaction + 1 %) at 35°—37° C. in 2 days.

The agar plates were all counted after two days’ incubation. Speaking generally, the number of colonies growing at 35° to 37° C. was found to be less than the corresponding number growing at 20° to 22° C., but the same gradual increase in the number of microbes during the manufacture of ice-cream is to be noted with both counts as the following summary shews (Table II).

Causes of the increase in the number of organisms in commercial ice-cream.

1. *Multiplication of organisms during cooling.* The average period of cooling before freezing was about $20\frac{3}{4}$ hours for the 50 samples under review and during this time the organisms not destroyed by the initial heating grow and multiply.

2. *The addition of organisms during the freezing process.* The high average count amongst the samples taken after freezing is also to be

TABLE II.
Classifying the results of Tests 1 and 2.

	Test 1. (Gelatine counts)			Test 2. (Agar counts)		
	"a"	"b"	"c"	"a"	"b"	"c"
	Samples taken immediately after heating	Samples taken after cooling	Samples taken after freezing	Samples taken immediately after heating	Samples taken after cooling	Samples taken after freezing
No. of samples shewing less than 100 organisms per c.c. ...	3	—	—	1	—	—
No. of samples shewing between 100 and 1000 organisms per c.c. ...	4	—	—	4	—	—
No. of samples shewing between 1000 and 10,000 organisms per c.c. ...	17	—	—	18	1	—
No. of samples shewing between 10,000 and 100,000 organisms per c.c. ...	9	10	2	15	15	6
No. of samples shewing between 100,000 and 1,000,000 organisms per c.c. ...	1	9	13	7	12	14
No. of samples shewing between 1,000,000 and 10,000,000 organisms per c.c....	2	5	5	5	11	10
No. of samples shewing between 10,000,000 and 100,000,000 organisms per c.c. ...	—	9	11	—	11	16
No. of samples shewing over 100,000,000 organisms per c.c. ...	—	2	7	—	—	4
No. of samples liquefied ...	14	15	12	—	—	—
Average no. of organisms per c.c. in samples taken immediately after heating ...	867,319			379,010		
Average no. of organisms per c.c. in samples taken immediately after cooling ...	13,042,857			6,861,600		
Average no. of organisms per c.c. in samples taken immediately after freezing ...	372,213,421			34,467,000		

TABLE III.
Shewing number of organisms in ice-cream immediately before and after freezing.

Manufacturer	Date of examination 1909	No. of organisms per c.c. capable of growing on nutrient gelatine (reaction +1%) at 20° to 22° C. in 3 days		No. of organisms per c.c. capable of growing on nutrient agar (reaction +1%) at 35° to 37° C. in 2 days	
		Immediately before freezing	Immediately after freezing	Immediately before freezing	Immediately after freezing
* No. 6	Feb. 28	10,000	30,000	230,000	470,000
„ 7	April 30	3,000	14,000	5,000	12,000
„ 26	May 1	2,300	36,000	18,000	29,000

* The nos. relate to laboratory references.

explained by the addition of microbes during the process of freezing by the dirtiness and carelessness of the manufacturer as already noted. This point is well illustrated by Table III, giving the results of the examination of samples taken from vendors immediately before and after freezing.

Experiment. A laboratory experiment was carried out to prove that it was possible to freeze without the introduction of microbes in excessive numbers. The ice-cream ingredients were duly boiled for 10 minutes and a looplet of the mixture was put into broth. No growth resulted. This sterile product was then poured while still hot into an ordinary freezer previously sterilised in the autoclave. The lid which had also been sterilised was put on and kept on, and the ice-cream mixture was frozen by rotation in a mixture of salt and ice. The process occupied 2 hours. The ice-cream was then stored in the laboratory store room, the only precaution that was taken being to keep the lid on. 5 minutes, 30 minutes, and 90 minutes after freezing a looplet was put into broth but no growth resulted. 24 hours afterwards, 1 cubic centimetre of the ice-cream, which had been kept frozen, was put into nutrient gelatine and found to contain 200 organisms.

3. *Multiplication of organisms during frozen period.* The increase in the number of organisms in commercial ice-cream is also due to multiplication of organisms during the frozen period, as shewn by the following experiment:

Experiment. On the 9th of April, 1909, ice-cream was obtained from a manufacturer in a freezer, which was brought to the laboratory and kept surrounded by ice and salt. On its arrival at the laboratory the "gelatine count" of the ice-cream was 327,000 colonies per cubic centimetre. After 3 hours the "gelatine count" was 388,000, after 6 hours 459,000, after 9 hours 603,000, and after 12 hours 611,000 colonies per cubic centimetre. Similarly the "agar count" of the ice-cream on its arrival at the laboratory was 93,000 colonies per cubic centimetre. After 3 hours the "agar count" was 112,000, after 6 hours 126,000, after 9 hours 130,000 and after 12 hours 139,000 colonies per cubic centimetre. During this experiment the temperature of the ice-cream varied between 28° F. and 28·8° F.

Test 3. The Bile-Salt Glucose Broth test.

All samples were submitted to this test, and the reaction produced within 48 hours noted. The quantities examined have already been given (vide Table I), the total for each of the "a" samples being 11·11

cubic centimetres, and for each of the “b” and “c” samples 11·1111 cubic centimetres. Only two samples gave a negative result. This is not as it should be, as ice-cream made in the laboratory—freezing being carried out immediately after boiling—produced “no change” in this medium after it had stood frozen 24 hours and 72 hours respectively, when as much as 20 cubic centimetres were examined.

The following table sets out the results of this test with the samples of the three series :

TABLE IV.

Shewing the results given by the three sets of samples with the Bile-Salt Glucose Broth test.

				“a”	“b”	“c”
				Samples taken immediately after heating	Samples taken after cooling	Samples taken after freezing
Producing no change in 11·11 c.c.	2	—	—
„ acid in 10 but not in 1 c.c.	3	—	—
„ „ 1 „ „ 1 c.c.	16	—	—
„ „ 1 „ „ 01 c.c.	20	10	1
„ „ 01 „ „ 001 c.c.	—	8	1
„ „ 001 „ „ 0001 c.c.	—	—	—
„ „ 0001 c.c.	—	1	1
„ acid and gas in 10 but not in 1 c.c.	1	2	—
„ „ „ 1 „ „ 1 c.c.	2	3	5
„ „ „ 1 „ „ 01 c.c.	5	7	5
„ „ „ 01 „ „ 001 c.c.	1	10	19
„ „ „ 001 „ „ 0001 c.c.	—	7	7
„ „ „ 0001 c.c.	—	2	11
Total				50	50	50

From Table IV it will be seen that nine of the samples taken immediately after heating produced the complete change—acid and gas—in varying quantities in this medium, one of these producing it in the smallest quantity put on, viz. 01 cubic centimetre. Thirty-one of the samples taken after cooling produced this reaction, two giving it in 0001 cubic centimetre. In the third series of samples, i.e. those taken after freezing, 47 out of the 50 samples examined shewed fermentation, no less than 11 producing acid and gas in 0001 cubic centimetre. The gradual increase in the number of samples producing this change, as well as in the number producing it in very small quantities of the ice-cream, is clear evidence of the contamination to which this article of food is subjected in the process of manufacture, and supports the previous results of the gelatine and agar counts (vide Table II).

The glucose fermenters.

The next part of Test 3, was the isolation and identification so far as possible of the organisms which produced acid and gas in Bile-Salt Glucose Broth. From the smallest quantity in each series shewing this change a plate was made on Bile-Salt Lactose Agar and the nature of the colonies developing in 24 hours at 35° to 37° C. noted. The colonies varied from red colonies with production of haze in the surrounding medium to red without haze, pink and lastly white. 95 such plates were made.

In six instances plates were made from different dilutions of the same sample, and in these six with one exception the red colonies with haze or red colonies, when present, were found in the lesser dilutions and the white colonies in the higher. So far as practicable three colonies were selected from each plate—two red and one white as a rule—and these were each subjected to 16 fermentation and other tests for the purposes of differentiation and classification.

In all 258 colonies were taken for examination from the 95 plates, 108 being red colonies with haze, 69 red, 22 pink and 59 white.

The differential reagents.

The reagents employed were:

For fermentation, glucose, lactose, mannite, maltose, galactose, laevulose, adonite, saccharose, raffinose, inulin, salicin, dulcite.

For fluorescence—neutral red.

For indol—peptone water.

For Voges and Proskauer's reaction—glucose broth.

For liquefaction—gelatine.

The composition of the media employed.

For fermentation. Where the fermenting power of an organism was to be tested gelatine media were employed having the following composition:

Sugar or alcohol	1 %	} tinted with litmus.
Peptone	2 %	
Lemco	1 %	
Gelatine	10 %	
5 % KHO Solution	1 %	
Distilled water	85 %	

For fluorescence: a medium similar in composition to the foregoing was used, litmus being omitted and neutral red replacing the sugar or alcohol so as to give a brilliant colour to the gelatine.

For indol:

Peptone	1 %
Salt	0.5 %
Distilled water	98.5 %.

For Voges and Proskauer's reaction:

Glucose	0.5 %
Peptone	1 %
Lemco	0.5 %
Distilled water	98.0 %.

The method of conducting the differential tests.

The method employed in carrying out these differential tests was based on that devised by Houston (1907, January, June).

As three colonies were selected from each plate a complete set of tubes for one operation was as follows:

Three of the following:

A. One 3" × 1" tube (white wool) containing five 2" × ¼" tubes with the following media:

1. Litmus glucose gelatine—white bead
2. „ lactose „ —brown „
3. „ mannite „ —blue „
4. „ maltose „ —green „
5. „ galactose „ —black „

Three of the following:

B. One 3" × 1" tube (blue wool) containing four 2" × ¼" tubes with the following media:

1. Litmus laevulose gelatine—no bead
2. „ adonite „ —red bead
3. „ saccharose „ —yellow bead
4. „ raffinose „ —indigo bead.

Three of the following :

C. One 3" × 1" tube (brown wool) containing four 2" × $\frac{1}{4}$ " tubes with the following media :

1. Litmus inulin gelatine—brown bead
2. „ salicin „ —blue „
3. „ dulcitol „ —green „
4. Neutral red „ —no „

One of the following :

D. One 3" × 1" tube (white wool) containing six 2" × $\frac{1}{4}$ " tubes with the following media :

- 3 with glucose broth (blue bead)
- 3 with peptone water (no bead).

After these tubes suitably marked were inoculated with the colonies to be tested they were allowed to incubate at 35° to 37° C., but at the end of three hours the gelatine media (A, B and C) were removed and placed for half an hour in an ice-chest and afterwards incubated at 20° to 22° C. The primary incubation at the higher temperature melts the gelatine, allows of some multiplication to take place and enables the organisms to distribute themselves throughout the medium. The production of acid or gas is readily indicated by the change of colour of the litmus or the presence of a bubble of gas in the medium.

The gelatine tubes were looked at daily for fermentation up to seven days. It was not found to be practicable on account of the numbers in use to keep them longer than that time. Definite production of acid and gas within that period was duly noted. No record of the degree of acidity or amount of gas production is possible by this method. At the same time it was clear that the organisms isolated, although yielding an unmistakably positive result, differed greatly in their powers of splitting the reagents employed. Houston (1907, December, pp. 23—25) has pointed out the extreme delicacy of gelatine media for fermentation tests and this statement was amply supported during the present inquiry, some organisms producing neither acid nor gas in seven days, others giving only a slight production of acid without gas, and others again a production of acid with only a bubble of gas.

It is also worthy of note that liquefaction of the gelatine may set in before fermentation has begun, making gas production impossible of recognition. This, however, did not happen in the case of any of the microbes investigated. But it raises the question if these gelatine sugar media are as useful for the differentiation of unknown microbes, as they

certainly are for the purpose to which Houston puts them, viz. the recognition of *Bacillus coli communis* and *Bacillus typhosus*, neither of which liquefy gelatine.

Paradimethylamidobenzaldehyde and persulphate of potassium were used for the indol test, the addition of 50 per cent. of the volume of peptone water of each of these giving the best results. Within the seven days allotted for the completion of these results it may be said that the intensity of the coloration depended upon the period of growth. But from the experience of a few instances specially tested it may be said that the indol reaction if not detectable at the end of 24 hours' incubation is not given later.

Voges and Proskauer's reaction was carried out in the following manner. Incubation in glucose broth was allowed to proceed for three days when there was added a quantity of a 2% solution of caustic soda equal in volume to about 25% of the volume of the broth. The tubes were then allowed to remain at room temperature for four days longer, daily observations being made during this time. The colour like that of a dilute alcoholic solution of eosin appeared as a rule within 24 hours, and in some instances went through varying shades to a pale green with a brown sedimentous deposit within the four days.

The value of the various reagents employed for differentiation.

258 colonies were examined, and from the results obtained it is possible to say that mannite, maltose, galactose and laevulose are useless for purposes of differentiation of *glucose fermenters* as all the organisms isolated produced acid and gas in those media. MacConkey (1905) has already made the same statement.

The position of lactose is interesting, and may be summed up thus:

(1) All colonies definitely red in colour on Bile-Salt Lactose Agar are lactose fermenters.

(2) Pink or white colonies on this medium may or may not split lactose. There were tested 22 pink and 59 white, and the majority of these produced lactose fermentation. MacConkey (1908, p. 324) has referred to this fermentation of lactose by colonies which are colourless on Bile-Salt Lactose Agar.

Fluorescence was produced by 233 out of 258 colonies examined, or by 90.3%. This is a higher percentage than obtained by the writer (1908, p. 16) when examining glucose fermenting organisms isolated from mussels. In this latter case the percentage was 50. A positive reaction as regards fluorescence is not always given by glucose-fermenters,

but the statement of Houston (1907, January, p. 47) that it is by the great majority is probably correct.

Indol was invariably produced by those red colonies producing definite haze in the surrounding medium, and it becomes questionable if it is valuable as a further test in these cases.

The position and exact interpretation of Voges and Proskauer's reaction have not yet been definitely decided. It is generally accepted as a reaction given only by bacilli of the *Bacillus lactis aerogenes* and *Bacillus cloacae* groups.

Fifty-four out of the 258 colonies examined, or 20·9 %, gave the reaction. It is not, therefore, a reaction given by every organism as MacL. Harris (1906, p. 250) has stated and MacConkey (1908) has already controverted. But of the 54 positive organisms only two could be placed in the *lactis aerogenes* group by their other reactions and only two more in the *cloacae* group. It is, therefore, difficult to place organisms in any particular group by means of this test. It seems rather to be a reaction given by a large number of organisms of different classes, although in the present state of our knowledge it may be well to accept it as a necessary qualification of the organisms belonging to the groups mentioned.

No particular mention requires to be made of the other reagents employed, which were all found to be more or less of diagnostic value. No great importance was attached to the test for liquefaction of gelatine, where the results were negative, as the time given (7 days) is short for this test. So far as it goes however it is interesting to note that 12 out of the 13 liquefiers were white colonies on Bile-Salt Lactose Agar.

The nature of the glucose fermenters isolated.

In only three of the ice-creams was it possible to isolate an organism giving the same reactions in two successive stages. This fact indicates the great multiplicity of *glucose fermenters* which abound in ice-cream and lends strong presumption to the view that fresh organisms are added by contamination during manufacture.

For the classification of these various isolated organisms it was necessary to put known organisms through the same tests in the same way. By the kindness of Drs R. M. Buchanan, A. C. Houston, Professor R. F. C. Leith, Dr A. T. MacConkey, Professor E. J. McWeeney and Dr W. G. Savage, the writer was able to obtain strains of various organisms, and the following table gives the reactions obtained with these bacilli as well as the sources from which they were got.

TABLE V.

Shewing the reactions of known organisms with the differential media employed in the present investigation.

Organism	Obtained from	Lactose	Fluorescence	Indol	Voges and Proskauer's reaction	Adonite	Saccharose	Raffinose	Inulin	Salicin	Dulcitol	Liquefaction of gelatine
<i>B. coli communis</i> (Escherich)	Dr A. T. MacConkey	+	+	+	-	-	-	-	-	-	+	-
<i>B. lactis aerogenes</i> ...	"	+	+	-	+	+	+	+	-	+	-	-
<i>B. acidi lactici</i> (Hüppe)	1. Kral through Dr R. M. Buchanan 2. Dr A. C. Houston 3. Dr A. T. MacConkey	+	+	+	-	+	-	-	-	-	-	-
<i>B. cloacae</i> ...	1. Kral through Dr R. M. Buchanan 2. Dr A. T. MacConkey 3. Dr A. C. Houston	-	+	-	+	-	+	+	-	-	-	+
<i>B. paracoli</i> (Widal) ...	Kral through Dr R. M. Buchanan	-	+	-	+	+	+	+	-	-	-	+
<i>B. enteritidis</i> (Gaertner)	1. Kral through Dr R. M. Buchanan 2. Dr A. C. Houston 3. Original strain through Dr W. G. Savage	-	+	-	-	-	-	-	-	-	+	-
<i>B. paratyphosus</i> B (Schottmüller) ...	1. Kral through Dr R. M. Buchanan 2. Dr A. C. Houston 3. Dr W. G. Savage	-	+	-	-	-	-	-	-	-	+	-
<i>B. paratyphosus</i> B (Achar)	Kral through Dr R. M. Buchanan	-	+	-	-	-	-	-	-	-	+	-
<i>B. paratyphosus</i> B (McWeeney)	Prof. E. J. McWeeney	-	+	-	-	-	-	-	-	-	+	-
<i>B. paratyphosus</i> A (Schottmüller) ...	1. Kral through Dr R. M. Buchanan 2. Dr A. C. Houston 3. Dr A. T. MacConkey 4. Dr W. G. Savage	+	+	-	-	+	+	+	-	+	+	-
<i>B. paratyphosus</i> A (Brian and Kayser)	Kral through Dr R. M. Buchanan	+	+	-	-	+	+	+	-	+	+	-
<i>B. aertrycke</i> ...	Dr A. T. MacConkey	+	+	-	-	+	+	+	-	+	+	-
<i>B. sinpestifer</i> ...	Prof. Uhlenhuth through Dr W. G. Savage	+	+	-	-	+	+	+	-	+	+	-
<i>B. psittacosis</i> ...	Dr A. T. MacConkey	+	+	-	-	+	+	+	-	+	+	-
<i>B. Friedlander</i> ...	Dr A. C. Houston	+	+	-	-	+	+	+	-	+	+	-
" (Nicolle)	Dr A. T. MacConkey	+	+	-	-	+	+	+	-	+	+	-
<i>B. levans</i> ...	"	-	+	-	+	-	+	-	+	+	-	+
<i>B. oxytocus perniciosus</i> ...	"	+	+	-	-	+	+	+	+	+	+	-
<i>B. rhinoscleromatis</i> ...	"	+	+	+	-	-	+	-	-	-	-	-
<i>B. Grünthal</i> ...	"	+	+	+	-	-	+	A	-	-	-	-
<i>B. coscoroba</i> ...	"	+	+	+	-	-	+	A	-	-	-	-
<i>B. cavicida</i> (Brieger)	"	+	+	+	-	-	-	-	-	A	-	-
<i>B. typhosus</i> ...	Prof. R. F. C. Leith	-	-	-	-	-	-	A	-	-	-	-

+ = production of acid and gas, presence of indol, presence of Voges and Proskauer's reaction or liquefaction of gelatine according to the column in which it appears.

- = absence of above according to column.

A = production of acid.

With the exception of the *Bacillus typhosus*, which produced merely acid, all the above organisms produced acid and gas in glucose, mannite, maltose, galactose and laevulose.

Comparing the reactions of the 258 *glucose fermenters* isolated from the samples of ice-cream examined with those given in Table V it was found that 66 of them could be classified as follows:

TABLE VI.

Classifying 66 of the 258 glucose fermenters isolated from the "a" "b" and "c" samples examined.

			No. of times recognised
<i>Bacillus oxytocus perniciosus</i> (V & P +)	5
„ „ „ (V & P -) or }	5
„ <i>rhinoscleromatis</i> (indol - inulin +) }	5
„ <i>coli</i> (sacc. + raff. + dulc. +)	8
„ „ („ - „ „ „)	4
„ <i>cloacae</i> (lact. + liquefaction -)	2
„ <i>coli</i> (sacc. + raff. - dulc. -) or }	3
„ <i>Grünthal</i> or <i>Bacillus coscoroba</i> }	3
„ <i>coli</i> (sacc. - raff. - dulc. -) or }	18
„ <i>cavicida</i> (dulc. -) }	18
„ <i>coli</i> (sacc. + raff. - dulc. +)	3
„ <i>acidi lactici</i> (raff. -)	4
„ <i>coli communis</i> (sacc. - raff. - dulc. +)	11
„ <i>Friedländer</i> (indol -)	1
„ <i>lactis aerogenes</i> (indol -)	2

Coli Group.

In Table VI we find that the *coli* group predominates, no less than 47 out of the 66 recognised organisms belonging to this group, 11 of these being the typical *Bacillus coli communis*. In only three instances did these members of the *coli* group not yield red colonies on Bile-Salt Lactose Agar and even these three were pink. It is interesting to note that 27 out of the total 47 *coli*-like microbes produced red surface colonies with haze in this medium. This indicates that while the colonies to be specially selected when using Bile-Salt Lactose Agar should be those producing haze other varieties must not be neglected in the search for *Bacillus coli*.

Organisms of this group have long been associated with contamination by faeces, human or otherwise. Doubtless they gain entrance to ice-cream from the dried particles in the court yards as well as from the makers' hands which are not washed before commencing its manufacture.

**Bacillus coli. Bacillus Grünthal. Bacillus coscoroba.
Bacillus cavitida.**

So far as these tests go it is impossible to distinguish between certain varieties of the *Bacillus coli*, *Bacillus Grünthal*, *Bacillus coscoroba* and *Bacillus cavitida* as seen on Tables V and VI. The *Bacillus Grünthal* has been placed in the coli group by Morgan (1905, p. 1258). *Bacillus coscoroba* is stated to have been the cause of an epidemic in swans. This was studied by Tritrop (1900, *Ann. Inst. Pasteur*, p. 224) and his results were published attributing the outbreak to this bacillus. The *Bacillus cavitida* has been isolated from faeces by Brieger (1884). He states that this organism is pathogenic for guinea-pigs when inoculated subcutaneously, but is without effect when taken with food. According to this observer *Bacillus cavitida* is allied to *Bacillus coli communis* and *Bacillus lactis aerogenes*.

Bacillus oxytocus perniciosus and Bacillus rhinoscleromatis.

Next to the coli group of organisms the organism found in most abundance was the *Bacillus oxytocus perniciosus*. This is an organism which was first described by Wyssokowitsch (Macé, 1904), who isolated it from old milk. It is pathogenic for mice and rabbits when large doses are used.

MacConkey (1906, p. 403) identified this organism 16 times in 170 organisms isolated from milk.

A glance at Table V will shew that it has been necessary to associate a variety of this bacillus as regards its fermentation tests with *Bacillus rhinoscleromatis*. Including both varieties here indicated the *Bacillus oxytocus perniciosus* has been found 10 times in the 258 microbes specially studied.

Bacillus cloacae.

The *Bacillus cloacae* has been isolated twice. A good account of this organism is given by MacConkey (1905, p. 348).

Bacillus lactis aerogenes and Bacillus pneumoniae (Friedländer).

The *Bacillus lactis aerogenes* has been identified twice and the *Bacillus pneumoniae* (Friedländer) once.

Authorities are not agreed as to the relationship between these organisms. Some consider them identical. Others consider the *Bacillus lactis aerogenes* as identical with the *Bacillus coli communis*. The presence of Voges and Proskauer's reaction is the most important test yet put forward for the identification of the *Bacillus lactis aerogenes*. MacConkey (1906, p. 403) isolated the latter twice from various samples of milk, but was unable to identify the *Bacillus pneumoniae* (Friedländer) during the investigation. He considered that the *Bacillus cloacae* and *Bacillus lactis aerogenes* could be found in milk in greater abundance after it had been kept some time.

Bacillus acidi lactici.

The *Bacillus acidi lactici* has also been recognised. MacConkey (1905, p. 378) has suggested that this organism while occurring in faeces sometimes disappears so quickly that, if found to do so constantly, it may provide an excellent test for the nearness or remoteness of pollution. In any case authorities are agreed that it is a close ally of *Bacillus coli communis* and as such must be regarded as evidence of serious contamination. It was found in two of the samples investigated.

Classification of the remaining organisms.

The reactions of the remaining 192 isolated organisms are shewn in Table VII.

In this table it is to be noted that the tests have been put down in the order in which they were conducted and the organisms giving the greatest number of positive reactions have been placed first on the table, the organisms with the greatest number of successive positive reactions taking precedence where the total number of positive reactions is the same.

In this way it has been possible to arrange the 192 unrecognised organisms in 74 different groups. Many of these groups approximate to the known organisms already described, but the differences are such as to prevent their inclusion in known groups. MacConkey (1905, 1906) lays considerable stress on the value of fermentation tests and the above results support his conclusions. The labour involved in working out these reactions for the various organisms is considerable and doubtless prevents this method of differentiation from being extensively used. It is equally true that in the present state of our

TABLE VII.

Classifying the 192 unrecognised glucose fermenters of the 258 isolated from the "a" "b" and "c" samples examined.

Total no. of organisms	Lactose	Fluorescence	Indol	Voges and Proskauer's reaction	Adonite	Saccharose	Raffinose	Inulin	Salicin	Dulcitol	Liquefaction of gelatine
2	+	+	+	+	+	+	+	+	+	+	-
3	+	+	-	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	-	+	+	-
5	+	+	+	-	+	+	+	+	+	+	-
1	+	+	+	-	+	+	+	+	+	-	-
2	+	+	+	-	-	+	+	+	+	+	-
2	+	+	-	+	-	+	+	+	+	+	-
1	-	+	-	+	+	+	+	+	+	-	+
1	+	+	+	+	+	-	+	-	+	-	-
6	+	+	+	-	+	+	+	-	+	-	-
1	+	+	+	-	+	+	+	-	-	+	-
1	+	+	+	-	+	+	-	-	+	+	-
1	+	+	+	-	-	+	+	-	+	+	-
1	+	+	-	+	+	-	+	-	+	+	-
6	+	+	-	-	+	+	+	+	+	-	-
3	+	+	-	-	+	+	+	-	+	-	+
7	+	+	-	-	-	+	+	+	+	+	-
3	+	-	-	+	+	+	+	-	+	+	-
4	+	-	-	-	+	+	+	+	+	+	-
2	+	+	+	-	+	+	-	+	-	-	-
2	+	+	+	-	-	+	+	-	+	-	-
3	+	+	-	+	+	+	-	+	-	-	-
2	+	+	-	+	-	+	+	-	+	-	-
4	+	+	-	-	+	+	+	-	+	-	-
2	+	+	-	-	+	+	-	+	+	-	-
1	+	+	-	-	-	+	+	+	+	-	-
3	+	+	-	-	-	+	+	-	+	+	-
1	+	-	+	+	-	+	+	-	+	-	-
1	+	-	+	-	+	+	+	-	+	-	-
1	-	+	-	+	+	+	+	-	+	-	-
1	-	+	-	+	-	+	+	-	+	-	+
1	-	-	-	+	+	+	+	+	+	-	-
1	-	-	-	+	-	+	+	+	+	+	-
4	+	+	+	-	-	+	+	-	-	-	-
1	+	+	-	+	+	+	-	-	-	-	-
2	+	+	-	+	-	-	+	-	+	-	-
1	+	+	-	-	+	-	+	-	+	-	-
15	+	+	-	-	-	+	+	-	+	-	-

TABLE VII (continued).

Total no. of organisms	Lactose	Fluorescence	Indol	Voges and Proskauer's reaction	Adonite	Saccharose	Raffinose	Inulin	Salicin	Dulcite	Liquefaction of gelatine
3	+	+	-	-	-	+	+	-	-	+	-
1	+	+	-	-	-	+	+	-	-	-	+
1	+	-	+	-	+	+	+	-	-	-	-
3	+	-	+	-	-	+	+	-	-	+	-
1	+	-	-	-	+	+	+	-	+	-	-
4	-	+	-	+	-	+	+	-	+	-	-
4	-	+	-	-	-	+	+	+	+	-	-
3	+	+	+	-	-	-	+	-	-	-	-
1	+	+	+	-	-	-	-	-	-	-	+
4	+	+	-	+	-	+	-	-	-	-	-
2	+	+	-	+	-	-	+	-	-	-	-
8	+	+	-	-	-	+	+	-	-	-	-
3	+	+	-	-	-	+	-	-	-	+	-
3	+	+	-	-	-	-	+	-	+	-	-
6	+	+	-	-	-	-	+	-	-	+	-
1	+	+	-	-	-	-	-	+	+	-	-
2	+	-	+	-	-	+	+	-	-	-	-
1	+	-	+	-	-	-	+	-	-	+	-
1	+	-	-	-	+	+	+	-	-	-	-
1	+	-	-	-	+	+	-	-	+	-	-
1	-	+	+	+	-	+	-	-	-	-	-
1	-	+	-	-	+	+	+	-	-	-	-
1	-	+	-	-	-	-	+	-	-	+	+
1	-	-	-	-	-	+	+	-	+	-	+
1	+	+	-	+	-	-	-	-	-	-	-
2	+	+	-	-	-	-	+	-	-	-	-
4	+	+	-	-	-	-	-	-	-	+	-
1	-	+	+	+	-	-	-	-	-	-	-
2	-	+	-	+	-	+	-	-	-	-	-
1	-	+	-	+	-	-	+	-	-	-	-
4	-	+	-	-	-	-	+	-	+	-	-
1	-	-	-	-	-	-	+	-	+	+	-
13	+	+	-	-	-	-	-	-	-	-	-
1	+	-	-	-	+	-	-	-	-	-	-
1	+	-	-	-	-	-	-	-	-	-	+
3	-	+	+	-	-	-	-	-	-	-	-

+ =production of acid and gas, presence of indol, presence of Voges and Proskauer's reaction or liquefaction of gelatine according to the column in which it appears.
- =absence of above according to column.
All the above organisms produced acid and gas in glucose, mannite, maltose, galactose and laevulose.

knowledge no organism can be absolutely recognised without such tests. It would serve a useful purpose if some definite method for their application and the classification of results was put forward for general adoption.

*Test 4. The test for the **Bacillus enteritidis sporogenes**.*

The next step in the inquiry was to ascertain in what numbers the spores of the *Bacillus enteritidis sporogenes* existed in the samples examined. The method of conducting this test was as follows. Fifty cubic centimetres of sterile water were put into sterile long necked flasks of 150 cubic centimetres capacity, and 100 cubic centimetres of ice-cream were added, and vaseline, into which a little hard paraffin had been put, was poured over in the usual way. Similarly 10 cubic centimetres of ice-cream were added to about 10 cubic centimetres of sterile water in a tube and sealed as above. In the case of 1 cubic centimetre of ice-cream this was added to about 10 cubic centimetres of sterile milk and water (half and half), the mixture of vaseline and hard paraffin being poured over as before to exclude the air. After heating to kill non-sporing organisms incubation was allowed to proceed at 35°—37° C. In the earlier samples examined sterile milk was used as above instead of water, but owing to the consistency of the ice-cream the typical *enteritidis change* was not well defined. The substitution of water for milk greatly improved the results and increased the ease of the manipulation as well.

A typical reaction resulted in many cases in 24 hours, but 7 days were allowed to elapse before a negative result was entered. Positive results were recorded only in those cases in which a typical torn and irregular pinkish clot was formed with a moderately clear whey and evolution of gas. Microscopic examination of the whey revealed the presence of large bacilli and spores when the conditions had become, by the forcing out of the vaseline, no longer anaerobic.

In no case was a smaller quantity of ice-cream than 1 cubic centimetre used, and the results are given in Table VIII.

This table shews that in only 9 of the 50 samples taken immediately after boiling was no reaction produced in all three quantities examined, viz. 100 cubic centimetres, 10 cubic centimetres and 1 cubic centimetre. All the other 41 samples shewed the *enteritidis change* in one or other of those quantities. It has already been pointed out that the ice-cream product should be a sterile fluid immediately after heating,

and in point of fact it was found that 100 cubic centimetres of milk, sugar and cornflour when heated and stored under laboratory conditions (vide p. 105) remained free from *Bacillus enteritidis sporogenes* for 72 hours.

TABLE VIII.

Classifying the results given by the three sets of samples with the Bacillus enteritidis sporogenes test.

	"a"	"b"	"c"
	Samples taken immediately after heating	Samples taken after cooling	Samples taken after freezing
Producing no change in 111 c.c. ...	9	4	2
Producing the "enteritidis reaction" in 100 but not in 10 c.c.	18	14	8
Producing the "enteritidis reaction" in 10 but not in 1 c.c.	19	21	17
Producing the "enteritidis reaction" in 1 c.c.	4	11	23
Total	50	50	50

The presence of the Bacillus enteritidis sporogenes in the ingredients of ice-cream.

So far as the ingredients of ice-cream are concerned the *Bacillus enteritidis sporogenes* was found in samples of milk and cornflour, but not in sugar. Milk, however, contains the organism in by far the highest numbers, for while the *enteritidis change* was given with as little as 0.1 cubic centimetre of milk, this reaction was not produced with less than 10 grammes of cornflour in the present investigation.

A consideration of the increase in numbers of the Bacillus enteritidis sporogenes.

(a) *By multiplication.* This is possible as the boiling and the layer of cream at the top render the conditions sufficiently anaerobic for the growth of this bacillus.

(b) *By addition from contaminated sources.* Organisms of this type are also added from dust and the sweepings of dirty court yards in which ice-cream is manufactured. Houston (1897—1900) has shewn that this organism is to be found in relatively great numbers in soil, and other observers have supported his conclusions, while Hewlett (1899) and Klein (1897—1899) have shewn that this organism is wide spread in its distribution and especially prevalent in dust.

Test 5. Streptococci.

Streptococci are considered by some bacteriologists to be indicators of recent pollution but the isolation of these organisms is not easy as is shewn by Gordon (1902—06), Houston (1903—05), Andrewes and Horder (1906), Andrewes (1906—07), Savage (1907—07), and my own experience (1908) confirms this. In one case in the present investigation I examined 67 minute colonies for streptococci without a single positive result. We have therefore in routine work to rely on the microscopical examination of a culture made from the material under investigation.

The results of such a microscopical examination are set out in the following table:

TABLE IX.

Classifying the results given by the three sets of samples with the streptococci test.

	"a" Samples taken immediately after heating	"b" Samples taken after cooling	"c" Samples taken after freezing
Shewing no streptococci in 11·11 c.c.	31	23	7
Shewing streptococci in 10 but not in 1 c.c.	—	1	2
„ „ „ 1 „ „ ·1 c.c.	3	—	1
„ „ „ ·1 „ „ ·01 c.c.	13	8	6
„ „ „ ·01 „ „ ·001 c.c.	3	15	7
„ „ „ ·001 „ „ ·0001 c.c.	—	3	15
„ „ „ ·0001 c.c.	—	—	12
Total	50	50	50

The sources of streptococci.

Streptococci have been found in enormous numbers in milk. In 31 of the samples of ice-cream taken immediately after boiling no streptococci were to be seen. It is therefore a fair conclusion that the original streptococci of the milk had been destroyed during the heating process. Only 7 of those 31 samples were still free from streptococci after freezing. In 24 samples therefore streptococci had entered. Streptococci are constantly found in faeces, manure, dust, and air, and from one or more of these sources of contamination, to which ice-cream is exposed, the 24 samples must have derived the organisms. It is here that more knowledge of the subject of streptococci is necessary. It is highly desirable

that we should be able to associate a particular variety of streptococcus with a particular source, and for this purpose isolation and rapid and complete differentiation are essential.

Suggested bacteriological standards.

In dealing with the subject of standards it is well to remember that a standard is not merely a matter of bacteriological average. It has to be considered also in the light of the attainable, and as a result of the experience of observers as regards the potential disease producing power of the material in question. The outbreaks recorded at the commencement of this paper clearly shew that ice-cream may be the carrier of many diseases. Unfortunately only in the outbreak investigated by Robertson (1906) was the implicated material submitted to bacteriological analysis, which however was conducted with a view to determining the causal agent and not the amount of pollution. If information on this latter point had also been provided, valuable data on which to base the consideration of standards would have been in our possession. Under these circumstances the question must be decided by the other two factors here mentioned.

A consideration of standards based on tests 1 and 2.

The average number of colonies capable of growing on nutrient gelatine or agar (vide Table II) in all these samples was high. This average cannot be taken as a fair test of what the standard of purity should be, for except in the cases of 7 samples taken immediately after boiling and found to contain less than 1000 organisms per cubic centimetre capable of growing at 20°—22° C., the initial heating could not have been satisfactory. If, on the other hand, these 7 samples be carefully considered at the three stages "*a*," "*b*," and "*c*," it is possible to arrive at a fair conclusion as to what should be the limit of the bacterial content of frozen ice-cream. Table X shews the results of enumeration so far as these seven samples are concerned.

It will be noted that the best of these samples are Nos. 42 and 46, with No. 41 not far behind. The sanitary conditions of the premises on which these were manufactured were fair. The premises of No. 2 were filthy, No. 50 dirty, No. 6 clean and No. 49 again fair. The high counts in No. 6 require explanation. This manufacturer shewed special care in the making of his ice-cream. He reserved buckets and an out-

house for its preparation. But this latter was situated in a close, confined, crowded, ill-paved yard common to two houses. The water-closet of the other tenant was out of repair at the time this sample was taken, and the ashbin a few feet away was full. Undoubtedly these insanitary and dirty arrangements close by combined with the long period of cooling and freezing—43 hours—contributed largely to the high counts obtained in the finished article. Another consideration is this, that the frozen sample was obtained from the manufacturer's employé off a trolley stationed in one of the busiest parts of Birmingham five hours after freezing had taken place. The dust of the traffic and the continual serving of customers in the open street by the not over clean seller must also have largely contributed to contamination and therefore high counts. Bearing all these facts in mind, and taking into consideration the figures in the seven samples set forth in Table X, it seems reasonable to assume that frozen ice-cream prepared under the various conditions laid down in the text should not contain more than 1,000,000 organisms per cubic centimetre capable of growing at 20° to 22° C. or 35° to 37° C.

TABLE X.

Shewing the number of organisms per c.c. in seven sets of samples, the "a" sets of which yielded less than 1000 organisms per c.c. capable of growing on nutrient gelatine at 20°—22° C. in 3 days.

Gelatine Counts.

	Sample no. 2*	Sample no. 6	Sample no. 41	Sample no. 42	Sample no. 46	Sample no. 49	Sample no. 50
"a"—after heating	less than 100	less than 100	200	600	less than 100	600	600
"b"—after cooling	180,000	liquefied	70,000	20,000	50,000	200,000	1,280,000
"c"—after freezing	7,000,000	12,000,000	100,000	50,000	100,000	2,720,000	4,600,000

Agar Counts.

	Sample no. 2*	Sample no. 6	Sample no. 41	Sample no. 42	Sample no. 46	Sample no. 49	Sample no. 50
"a"—after heating	200	2,000	900	200	less than 100	6,600	3,000
"b"—after cooling	20,000	100,000	70,000	10,000	„ 10,000	80,000	880,000
"c"—after freezing	5,000,000	20,000,000	600,000	30,000	20,000	140,000	2,000,000

* The numbers of the samples relate to laboratory references.

The experiment which was carried out (vide p. 105) shews that it is possible to manufacture ice-cream in the laboratory, which does not shew more than 200 organisms per cubic centimetre after standing frozen 24 hours. It is impossible for manufacturers to work under laboratory

conditions, but they can readily prepare ice-cream which will pass the standard of 1,000,000 indicated. This is shewn by the results of the examination of ice-cream prepared by manufacturers under my instructions as follows:

Method of preparation. The ice-cream mixture was boiled in a pot directly over the fire for 15 minutes, in the case of manufacturers Nos. 6, 7 and 26 in Table XI, and afterwards poured into the freezer through a metal sieve. In the case of the other manufacturers, Nos. 1, 11 and 12 in Table XI, the so-called "water-bath" method was used in which the freezer containing the ice-cream mixture was put into cold water in a large pot or boiler. The water was brought to the boil and kept boiling for 30 minutes (Nos. 1 and 11) and for 45 minutes (No. 12) respectively.

In each case freezing was carried out immediately after heating.

All the utensils used were thoroughly cleansed with soda and hot water and scalded with hot water immediately before use.

The manufacturer's hands and arms were carefully scrubbed and washed.

The following table gives the results:

TABLE XI.

Manu- facturer	Time of taking sample	No. of colonies per c.c. capable of growing on nutrient gelatine (reaction +1%) at 20° to 22° C. in 3 days	No. of colonies per c.c. capable of growing on nutrient agar (reaction +1%) at 35° to 37° C. in 2 days
*No. 6	Immediately after heating	1,200	10,000
	" " freezing	3,300	16,000
	22 hours " "	27,000	41,000
	92 " " "	119,000	127,000
No. 7	Immediately after heating	2,000	11,000
	" " freezing	2,000	16,000
	22 hours " "	14,000	48,000
	70 " " "	77,000	110,000
No. 26	Immediately after heating	less than 1,000	less than 1,000
	" " freezing	" "	" "
	20 hours " "	4,500	6,000
	44 " " "	41,000	35,000
No. 1	Immediately after heating	less than 1,000	3,000
	" " freezing	1,100	4,000
	48 hours " "	63,000	71,000
	62 " " "	112,000	125,000
No. 11	Immediately after heating	sterile	sterile
	" " freezing	200	400
	26 hours " "	9,000	16,000
	46 " " "	30,000	26,000
No. 12	Immediately after heating	sterile	sterile
	" " freezing	200	400
	20 hours " "	7,000	10,000
	44 " " "	35,000	17,000

* The nos. relate to laboratory references.

From Table XI it will be seen that the results obtained immediately after heating by the water-bath method (Nos. 1, 11 and 12) are better than those obtained when the mixture is boiled directly over the fire. This is due to the fact that during heating by the latter method constant stirring with the exposure of a large surface has to be practised to prevent the material being burnt, and this does not conduce to obtaining a sterile article.

The Board of Public Health of the State of Victoria (1906) require that ice-cream shall not contain more than 50,000 organisms per cubic centimetre. But this standard is probably too severe considering the fact that in an investigation conducted in Philadelphia by Pennington and Walter (1907, p. 1016), a certain manufacturer "endeavoured to preserve the strictest cleanliness possible," and yet produced ice-cream with organisms varying in number from 6,535,000 to 35,120,000 per cubic centimetre. The standard of 1,000,000 here laid down may be called lenient, yet it condemns 35 or 70 % of the samples examined.

That this standard is reasonable and easily attained, if the ice-cream is properly manufactured and not stored for too long a period (which should not be longer than 48 hours after heating), is clearly shewn by Table XI.

A consideration of a standard based on Test 3.

None of the seven samples which were taken immediately after boiling and found to contain less than 1000 organisms per cubic centimetre and which have been discussed in "A consideration of standards based on tests 1 and 2," produced acid and gas in Glucose Bile-Salt Broth in any of the quantities examined.

The ice-cream which was manufactured as above by manufacturers Nos. 6, 7, 26, 1, 11 and 12 produced acid and gas as follows:

No. 6—after freezing 92 hours:

Present in 10 c.c. and 1 c.c.

No. 7—after freezing 70 hours:

Present in 10 c.c. and 1 c.c.

No. 1—after freezing 62 hours:

Present in 10 c.c. and 1 c.c.

The complete reaction was not given by any of the other samples prepared as described by these six manufacturers.

Ice-cream prepared in the laboratory (vide p. 106) after 72 hours' freezing failed to shew the presence of *glucose fermenters* in as much as 20 cubic centimetres of the ice-cream.

Bearing these facts in mind it is not too much to require that ice-cream prepared with due observance of the various conditions laid down should not contain *glucose fermenters* in less than 0·1 cubic centimetre of the finished product. In fact this is a lenient standard. Yet on this basis only 13 or 26 % of the samples examined would be passed—a fact in strong support of the uncleanly conditions under which this article is manufactured.

A consideration of a standard based on Test 4.

In the case of the ice-cream prepared as described by the six manufacturers, Nos. 6, 7, 26, 1, 11 and 12, the *Bacillus enteritidis sporogenes* was found after the mixture had stood frozen in all cases in 100 cubic centimetres and in three instances in 10 cubic centimetres as well. In no case was it found in 1 cubic centimetre.

Ice-cream which was prepared in the laboratory (vide p. 119) and afterwards allowed to stand frozen and covered for 72 hours did not shew the *enteritidis change* when 100 cubic centimetres were examined.

It is further interesting to observe that in two of the fifty frozen samples originally examined this change was not given in 111 cubic centimetres.

With these facts before us it is allowing a wide margin for unavoidable accident to state that a well made and properly stored ice-cream should not shew the presence of this bacillus in less than 10 cubic centimetres. Houston (1905) states that a sample of milk immediately cooled and maintained at a temperature of 10° C. should be objected to if it gives the *enteritidis change* in less than 1 cubic centimetre. Orr (1908) supports this standard. In only 2 out of 75 samples of milk was he able to find this bacillus in a less quantity than 1 cubic centimetre. If these observers reckon this a fair standard for milk merely cooled and kept cool it is not unfair to ask for the higher standard of 10 cubic centimetres for milk, sugar, and cornflour, which is first boiled and immediately frozen and kept frozen. On this standard 27 or 54 % of the samples of ice-cream examined were satisfactory.

A consideration of a standard based on Test 5.

In the light of our present knowledge as regards streptococci it is a difficult matter to set up a definite standard. The difficulty is increased by the consideration of an experiment in the manufacture and storage of ice-cream conducted in the laboratory under the same conditions as

similar experiments already quoted. In the mixture thus prepared and stored streptococci, although absent immediately after boiling made their appearance in 20 cubic centimetres within 24 hours. In the same time neither the presence of *glucose fermenters* nor the *Bacillus enteritidis sporogenes* could be demonstrated in the same amount.

In the ice-cream specially prepared by manufacturers, Nos. 6, 7, 26, 1, 11, 12, streptococci were present in .01 cubic centimetre in the frozen samples of four of these makers and absent from 10 c.c. in the samples of the two remaining manufacturers. In no case were they present in .001 cubic centimetre.

Keeping these results in view I am not disposed to urge in the present state of our knowledge regarding the significance of streptococci that they must be absent from large amounts of frozen ice-cream, and would suggest that ice-cream be accepted which does not shew their presence in less than .001 cubic centimetre. On this standard 38 or 76 % of the samples examined pass. Houston (1905) for like reasons suggests a lenient standard as regards streptococci in milk. He suggests that the presence of this class of organism in less quantity than .0001 cubic centimetre lays the milk open to objection from the bacteriological standpoint. In asking for a higher standard in the case of ice-cream it is because it is comparatively easy to prepare ice-cream which is initially sterile, while it is a difficult matter to procure freshly drawn milk which does not shew the presence of these organisms.

SUMMARY.

(1) The premises of 50 manufacturers of ice-cream were inspected, their methods investigated, and bacteriological examinations made of samples taken

“a” immediately after heating,

“b” after cooling,

“c” after freezing.

(2) The trade is not carried on under the conditions or with the precautions necessary to secure a clean product.

The sources of the contamination of ice-cream.

(3) Bacteriologically polluted ice-cream is due to

(A) Insufficient initial heating;

(B) Contamination during cooling and freezing from

- (a) unclean vessels and covers,
- (b) the addition of unclean ice to hasten freezing,
- (c) the unclean hands of the manufacturer,
- (d) dirty surroundings.

The scientific method of the manufacture of ice-cream.

(4) To secure a pure ice-cream :

(a) All vessels should be thoroughly cleansed immediately before use and reserved for the manufacture of ice-cream. They should be stored in a clean place.

(b) The manufacturer's hands and forearms should be thoroughly scrubbed and cleansed before each stage of the process. The clothing likely to come in contact with the ice-cream should also be clean.

(c) Fresh milk should be used in its manufacture.

(d) The ingredients should be boiled directly over a fire for ten minutes, or heated by means of a water-bath at boiling point for 30 minutes. The latter method is the better as the former is liable to burn the mixture.

(e) The mixture should be frozen, immediately after boiling preferably in a freezer of the American pattern. Thereafter the ice-cream should be kept frozen while in the vendor's possession.

(f) No ice-cream should be exposed for sale 48 hours after boiling.

(g) The premises on which ice-cream is manufactured should be approved and registered by the local authority and should be constantly supervised.

Bacteriological standards.

(5) Ice-cream made under the conditions laid down in (4) :

(a) Should not contain more than 1,000,000 organisms per cubic centimetre capable of growing on nutrient gelatine (reaction + 1 %) at 20°—22° C. in 3 days.

(b) Should not contain more than 1,000,000 organisms per cubic centimetre capable of growing on nutrient agar (reaction + 1 %) at 35°—37° C. in 2 days.

(c) Should not produce acid and gas in Bile-Salt Glucose Broth with a less quantity than 0.1 cubic centimetre.

(d) Should not contain the *Bacillus enteritidis sporogenes* in less than 10 cubic centimetres.

(e) Should not contain streptococci in less than .001 cubic centimetre of ice-cream.

The isolated organisms.

(6) Two hundred and fifty-eight *glucose fermenters* were isolated and studied.

(a) 66 of these were recognised as follows:

One or other variety of *Bacillus coli* was recognised 47 times.

The *Bacillus oxytocus perniciosus*, or the *Bacillus rhinoscleromatis* was recognised ten times.

The *Bacillus acidi lactici* was identified four times.

The *Bacillus cloacae* was isolated twice.

The *Bacillus lactis aerogenes* was identified twice.

The *Bacillus pneumoniae* (Friedländer) was isolated once.

(b) It was possible to arrange the remaining 192 organisms in 74 different groups.

The work in this paper was done while I acted as Assistant Medical Officer of Health to the City of Birmingham, and I have to thank Dr John Robertson, who suggested the subject to me, for his help and guidance during the inquiry and for permission to publish this paper.

The bacteriological work was conducted in the Pathological Department of the University of Birmingham by kind permission of Professor R. F. C. Leith.

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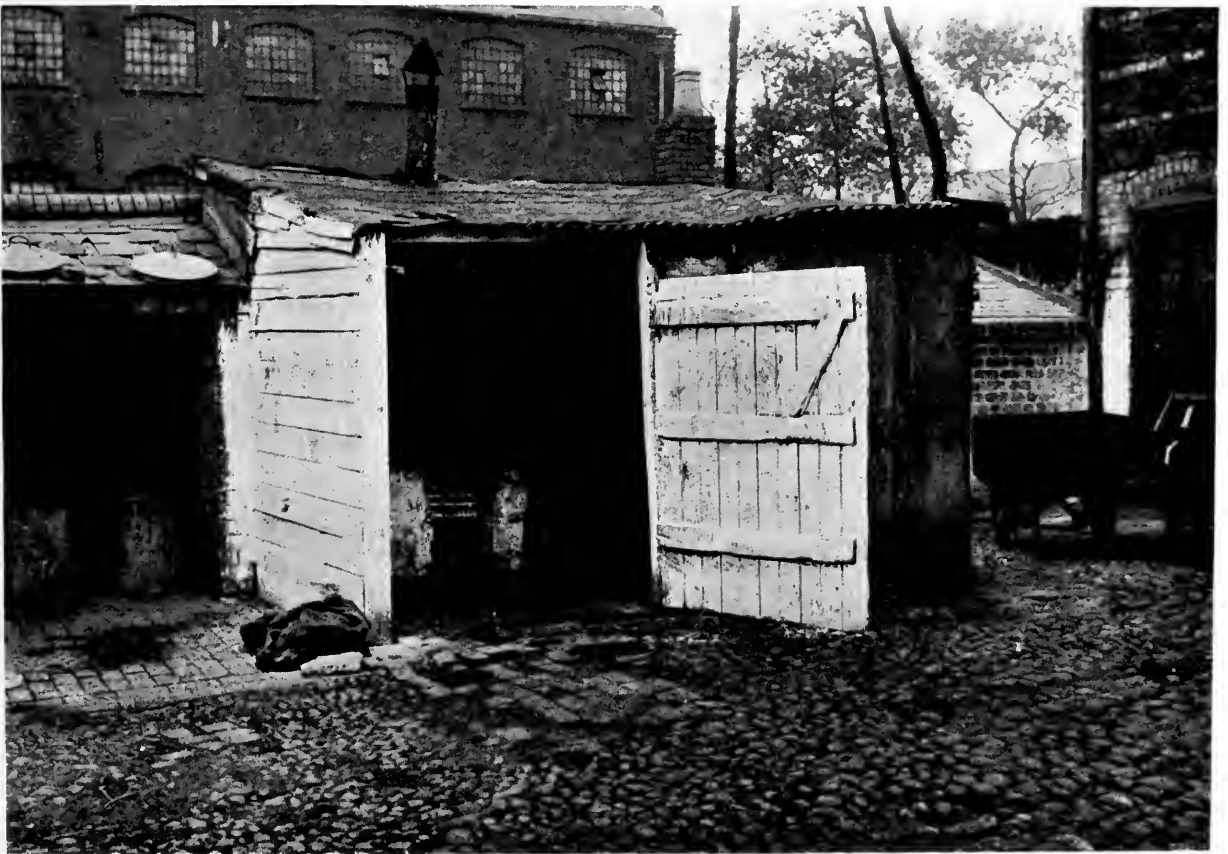
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PLATE VII.

Photograph. A common type of premises. Classified as "dirty" and used for the preparation of ice-cream by eight manufacturers. Note the roof, ill-fitting boards, unpaved floor, roughly paved yard and uncovered dustbins close by.



INTERNATIONAL HYGIENE EXHIBITION DRESDEN 1911.

MAY—OCTOBER.

INVITATION TO EXHIBIT IN THE SCIENTIFIC SECTION.

The essential preparations for the International Hygiene Exhibition Dresden 1911 are finally completed.

The Exhibition will include five extensive sections: the Scientific, the Historical, the Popular Sections, the Section of Sports, and, inseparably connected with them all, Industry.

The **Scientific Section**, which will be explained further on in greater detail, aims to present as completely as possible a universal picture of the science of hygiene.

The **Historical Section*** will exhibit the history of hygiene as a connected whole from the earliest ages to the beginning of the preceding century.

The **Popular Section**, an enlarged presentation of the special Exhibition, "Infectious diseases and their prevention," held in Dresden in 1903 at the German City Exhibition, is to include the whole province of the hygiene of the individual. The attempt shall be made to give the great mass of people a clear conception of the nature of the human body and of the functions of its various organs; it shall also be plainly demonstrated to every individual that he is able under certain circumstances not only to maintain the condition of his health and strength,

* Special publications concerning the Historical Section, the Sports Section, and Industry will be sent on request.

but also to improve it; further, the people shall be aroused to a consciousness of the significance of legislative measures enacted in the interest of health protection.

In the **Sports Section*** there will be, in connection with the Scientific Exhibition, a practical demonstration of all classes of sports; moreover, these demonstrations will be utilized in such a way that a most thorough study of the influence of physical exercise upon the human organism will be conducted in a special laboratory according to the very latest improved methods.

Industry*, in so far as it is not already represented in combination with the exhibitions of the above mentioned departments, will form an independent section; the exhibition rooms will be as closely connected as possible with those of the corresponding scientific groups.

We now take the liberty of respectfully inviting you to exhibit in the Scientific Section of the Exhibition.

The enclosed publications will give you further details as to the organization, the programme, the classification, the scope etc. of the Exhibition.

The conditions for the Scientific Section of the Exhibition will make it quite plain that exhibitors in this division incur no special expense for space rent, for the general architectural arrangement of the Exhibition rooms, for the insurance of exhibits against fire, burglary, and damage or loss during transportation, as well as for surveillance of the exhibits.

It is a fundamental principle of the Scientific Section of the Exhibition that all exhibits shall be separated according to groups and installed in rooms according to the Exhibition programme, the arrangement consequently being determined by the material to be exhibited, and not by the exhibitor.

This circumstance necessitates a careful separation of the proposed exhibits by the Group Presidents and the Administration. As this separation must be made according to the statements in the application forms, we respectfully beg you to fill out these forms as carefully as possible.

* Special publications concerning the Historical Section, the Sports Section, and Industry will be sent on request.

Further, we should not neglect to point out that, in conformity with our plan of adhering to a uniform, finished demonstration of the separate groups, and of avoiding wearisome repetitions, it will perhaps not always be possible to accept unconditionally all exhibits (cf. § 5 of the Exhibition Conditions).

Plastic exhibits (original products, in case of larger articles, models) are particularly desired. Special value is also laid on articles to be exhibited in operation.

Collective exhibits, in the case of which the articles of one exhibitor are installed together in the same room, can, as is shown in the preceding remarks, be permitted only by exception, when there are particularly cogent reasons for such a course. In such exceptional cases special arrangements must be made with the Administration, the question of first importance being the adjustment of expense.

In accordance with the fundamental idea which has already been explained in detail, and which is to be the standard in all decisions of the Scientific Section of the Exhibition, this section, which aims solely to show the scientific foundations of its separate branches, can admit industry only to such a degree as is necessary to give an adequate conception of the present condition of science. Small objects (e.g. apparatus, instruments, laboratory equipments) may be exhibited in the original; on the other hand, industrial products of greater size may be exhibited only in the form of models. All industrial products which are exhibited for market purposes, or those in which the possibility of advertising influences can be seen, are subject to the conditions for exhibiting manufacturers; in particular, a space fee is charged in such cases. On the other hand, when the manufacturers are not named, and when the origin of the industrial products cannot be recognized, facts which consequently exclude all business intentions or advertising possibilities, these wares may be regarded as exhibits in a scientific sense; such may be admitted to the Exhibition according to the conditions for the Scientific Section.

It is assumed that **foreign states** will arrange special exhibitions with which, as a rule, institutions and individual exhibitors of the country in question might be associated; but of course foreign exhibitors are also at liberty to display their exhibits in the various groups of the Scientific Section.

For each of the 44 groups and sub-groups of the Scientific Section, 200 square metres of ground space are provided on an average. However, if necessary, even greater space can be supplied, subject to the consent of the Administration.

We take the liberty of asking you to enter in the application form the articles which you consider suitable for exhibition, and we should be grateful to you if you would send in your application as early as possible, in order that the protracted and extensive preparations for the reception and installation of the exhibits may be completed on time (application to be made before July 1, 1910).

When the term for making application has expired, a decision will immediately be taken concerning the admission of the proposed exhibits.

INTERNATIONAL HYGIENE EXHIBITION DRESDEN 1911.

Directorium :

K. A. LINGNER,
Geheimer Kommerzienrat,
Dresden.

DR RENK,
Geheimer Medizinalrat, Professor of Hygiene,
Director of the Central Office for Care of the
Public Health, Dresden.

General Secretary for the Scientific Department :

DR WEBER,
Regierungsrat, Member of the Imperial Board of Health, Berlin.

(The foregoing notice is published by request of the Committee of Management of the International Hygiene Exhibition, Dresden. Ed.)

INVESTIGATIONS ON TYPHUS FEVER¹.

BY PROFESSOR CH. NICOLLE.

Director of the Pasteur Institute of Tunis.

(Author's Abstract.)

NICOLLE, CH. Reproduction expérimentale du typhus exanthématique chez le singe. *Comptes-Rendus de l'Académie des Sciences*, séance du 12 juillet 1909.

NICOLLE, CH., COMTE, CH. et CONSEIL, E. Transmission expérimentale du typhus exanthématique par le pou du corps. *Comptes-Rendus de l'Académie des Sciences*, séance du 6 septembre 1909.

NICOLLE, CH. Recherches expérimentales sur le typhus exanthématique entreprises à l'Institut Pasteur de Tunis pendant l'année 1909. *Annales de l'Institut Pasteur*, avril 1910.

Ces recherches, les premières qui aient été entreprises avec succès sur cette maladie, ont montré :

1. La sensibilité du chimpanzé au virus humain.
2. La sensibilité du *Macacus sinicus* (bonnet chinois) au même virus après passage par le chimpanzé.
3. La sensibilité du bonnet chinois au virus du bonnet chinois. Cependant le virus semble perdre assez rapidement son activité et ne plus donner après quelques passages qu'un typhus abortif.
4. La virulence du sang prélevé dès le début de l'infection et jusqu'au moment de l'éruption (chez l'homme et l'animal).
5. La résistance du bonnet chinois et du *Macacus cynomolgus* au virus, lorsque celui-ci provient directement de l'homme ; et la résistance du *M. cynomolgus* et du *M. rhesus* au virus du bonnet chinois.
6. L'absence de pouvoir immunisant du sang humain non typhique pour le bonnet chinois.
7. Le pouvoir immunisant du sang humain typhique (non infectant) pour le bonnet chinois vis à vis du virus de passage.

¹ Communicated by request of the Editors. G. H. F. N.

8. La transmission du typhus du bonnet chinois infecté au bonnet chinois neuf par l'intermédiaire du pou du corps humain (*Pediculus vestimenti*). L'incubation chez l'animal est longue (40 jours dans les deux expériences où elle a été réalisée); elle paraît généralement plus courte chez l'homme.
9. Une nécrose des globules polynucléaires neutrophiles du sang.

Deux de ces points sont d'importance capitale: la reproduction expérimentale du typhus chez le singe qui va permettre l'étude scientifique de l'infection; sa transmission par le pou du corps qui indique les règles à suivre pour sa prophylaxie.

EPIDEMIC GENERALISED VACCINIA.

By ERNEST HILL, M.R.C.S. Engl., L.R.C.P. Lond.,
D.P.H. Camb.,

Health Officer for the Colony of Natal,

AND G. A. PARK ROSS, M.B., BAC.SURG. Edin., D.P.H.,
R.C.P.S. Edin.,

District Health Officer, Ngutu.

THE occurrence of a general cutaneous eruption consequent upon vaccination, that is to say the inoculation of vaccine virus upon an abraded skin surface, is a possibility generally recognised. But the meagre literature of the subject, and the scanty and brief references in present-day text-books of medicine indicate that the condition is only occasionally encountered.

In Nothnagel's *Encyclopedia* (American edition) a few lines are given to "accidental symptoms" classed as (1) macular erythema, (2) accessory pocks due to auto-inoculation; and a brief allusion is made to a vesicular or bullous eczema following vaccination.

In Allbutt and Rolleston's *System of Medicine*, under Vaccination in Man—a Clinical Study, Acland enters into detailed descriptions of vesicular eruptions, which, following Longet, he groups as (1) spontaneous eruptions, (2) eruptions generalised by auto-inoculation. It appears, however, that he does not regard them as of much practical importance.

In Osler's *System of Medicine*, under Irregular Vaccinations, three conditions are described: (*a*) local variations, (*b*) generalised vaccinia, (1) vesicles in the neighbourhood of the primary vesicle—not uncommon, (2) a true generalised pustular rash, beginning between the eighth and fourteenth days, in which secondary pocks may continue to appear for five or six weeks. This, it appears, may prove fatal in children but is less common than (1).

The rarity of generalised vaccinia is also indicated by the fact that in the six years 1902—1908 over five hundred thousand persons, mostly

natives, have been vaccinated in Natal with glycerinated calf-lymph, but nothing of the sort has been reported.

The occurrence, then, in epidemic incidence, of febrile illness and constitutional disturbance associated with general vesicular eruption, following upon vaccination with glycerinated calf-lymph, appears to be sufficiently important to merit the attention of those interested in preventive medicine.

Before proceeding further it is well to make a brief statement of the circumstances in which natives are vaccinated, so that the reason for lack of precision in some statements may be appreciated. With six exceptions all cases reported have been of natives.

All vaccination in Natal is done with calf-lymph, which must, in accordance with law, be issued or approved by the Health Officer for the colony. Arm to arm vaccination is prohibited. The magistrate in each district arranges certain places as centres convenient for assembling of natives, which are in due course visited by the district surgeon. Many of the centres, to which children are brought from as far as ten miles, are twenty miles or more from the magistracy, and in consequence nothing is known as to the results unless the natives themselves complain or report to officials, missionaries or storekeepers. Thus a few isolated cases of unusual sequelae to vaccination would pass unnoticed; but if the cases were numerous or the illness severe the matter would be reported and the possibility of such an occurrence following vaccination would quickly become known, after which even insignificant cases would be brought to notice.

It cannot therefore be concluded that because no reports have been received from any one district that no such results have ensued at all, but it certainly may be taken that such occurrence was exceptional.

The eruptive manifestations may roughly be divided into three classes:

I. In twenty per cent. a generalised eruption appeared simultaneously all over the body, from about the fourteenth to the twenty-eighth day after vaccination, in many instances after the scab had fallen off.

II. In seventy-five per cent. secondary vesicles appeared round the original vesicles on about the eighth day, followed by crops in various parts of the body for the next two months, in some cases even for three months.

III. In the remainder an eruption followed one of the preceding types, then subsided altogether, to reappear a month or two later after some ailment or a burn.

The vaccination scab in a few instances persisted over the twentieth day, but the local reaction was seldom severe. In group I it usually separated before the eruption appeared; in about a third of the cases in group II it persisted longer, usually with some induration round it but always separated before the crops of pocks ceased to appear. In many instances an impetiginous condition appeared round the scab but was very amenable to treatment.

The generalised eruption of group I appeared as small macules surrounded by a slightly injected area, the redness disappearing on pressure. The hard, shotty feeling so characteristic of the papule of variola was not observable. The child would be petulant and feverish. In two or three days the macules were succeeded by vesicles, at first the size of a millet seed, which in six days attained the size of a nux vomica seed. Umbilication was frequently noted. If the vesicles were broken a watery or a reddish-stained jelly-like matter exuded. About the eighth day they became pustular, and secondary fever supervened; the itching appeared to be severe.

The eruption affected the buccal mucous membrane, gums, palate and pharynx and appeared particularly on forehead, neck and crown, where impetiginous crusts were formed. It was marked and in many cases confluent on the front of the neck; less on thorax and back, and scanty or absent from the abdomen; marked and often confluent on buttocks, and generally more extensive on the vaccinated than upon the other arm. It affected the palms almost always; was profuse as a rule on thighs and ankles, and in a few cases was observed on the soles. Two to three days were generally occupied in appearance of the eruption, which came out more or less in the order given above. The general appearance of a well-established case was that of a varioloid.

The limited eruption of class II began round the vaccination marks on or about the eighth day. It followed much the same line of distribution as the above, but its appearance was irregular and in some instances it continued for three months, crops of vesicles coming out in various situations, many healing up before others appeared. These in a number of cases only occurred in situations which admit of scratching, but in certainly as great a number were not so distributed, although there was a predilection for moist and warm spots, as the buttocks or points exposed to the chafing of garments, as the intrascapular region and the neck. For some children, particularly when digestion was disordered, a fine scaly condition was observed and in these the eruption

was always more severe but diminished when digestive disorders were corrected.

All the fatal cases seen, and many others with severe general eruption, suffered from broncho-pneumonia during the course of the illness or as a terminal affection. Diarrhoea was a feature of some and was more common in the later stages when, notwithstanding that the ulcerated surfaces, although extensive, were clean, the child passed into an asthenic condition and died of exhaustion. Deaths occurred only in late stages of the condition, the earliest five weeks after vaccination, and some as late as three months. The cause appeared to be septic absorption and no death was noted to have occurred until the eruption had been pustular for two weeks.

Class III comprised a few abnormal cases of which the following is an example :

Child aged two years, vaccinated May 4th and subsequently affected with mild general vaccinia of the type of class II. By the middle of June fresh pocks had ceased to appear. Early in July the child was severely scalded, and an area of foul suppuration of some twenty-two square inches resulted. One week later about thirty pocks similar to those of vaccinia appeared. General symptoms of serious illness supervened and death occurred in twenty-four hours.

We have found no evidence that the disease has been transmitted from person to person.

It is so obvious that the disease is a specific entity that the question of differential diagnosis needs not to be entered upon. If the cases had been few, or not confined to persons freshly vaccinated it might have been necessary to distinguish between it and varioloid or to consider as a possibility foot and mouth disease, impetigo contagiosa, or syphilis, but the epidemic incidence in vaccinated persons only precludes the possibility of error in respect to any of these.

Lymph from a vesicle on the foot of one child was inoculated upon a calf. The bacteriologist reported that it produced a line of "typical but not vigorous vesicles."

In respect to what may be termed the *epidemiology* it is to be noted that the illness in all cases ensued upon the use of lymph obtained from one source only, and that about three-fourths of the persons attacked were vaccinated from a parcel bearing one numerical index, the remainder from parcels indicated by four other numbers.

In round numbers 45,000 persons were vaccinated in the cool season

of 1909, the lymph used being obtained from three sources. Two parcels were received from the Jenner Institute sufficient for eight thousand and sixteen thousand vaccinations respectively, the first bearing only the one number 4698 and the second five numbers. Lymph 4698 was sent out to eleven districts.

The second parcel was designated by five numbers: 4782, 4785, 4786, 4787, 4788. It was distributed to fifteen districts and about thirteen thousand vaccinations were effected from it. The lymphs were used mainly for vaccinations of natives, but a few hundred Indians and a score or so of Europeans were also vaccinated with them.

A notable feature in the incidence of vaccinia is that six-sevenths of all cases occurred in up-country districts, and one-seventh only in the coastal area, although three-fifths of the lymph was used in the latter. This is particularly marked in the case of 4698, after the use of which in three out of four up-country districts the disorder was epidemic, affecting in greater or less degree about fifteen per cent. of those vaccinated, numbering about two thousand three hundred, although in the fourth district five cases only were recorded. In four of the other districts no case was notified, and in the remaining three four cases were reported. Thus, while in the up-country districts as a whole the incidence was about one in eight of persons vaccinated with lymph from this source, in the warmer districts it was less than one in one thousand. In several of the warm districts the lymph was reported to have given a weak reaction and a low percentage of successful results.

TABLE.

Showing the number of Districts to which lymph from several parcels was issued, and the number of cases and of deaths which were reported.

numbers :—	4782 & 4785				4786				4787 & 4788				4698				Total	
	Number of Districts	Issue	Cases	Deaths	Number of Districts	Issue	Cases	Deaths	Number of Districts	Issue	Cases	Deaths	Number of Districts	Issue	Cases	Deaths	Cases	Deaths
Districts	6	1452	0	0	4	346	50	1	6	2700	13	0	4	2946	300	27	363	28
Districts	6	4000	50*	1	6	960	2	0	6	3000	6	2	7	4550	4	0	62	3
	12	5452	50	1	10	1306	52	1	12	5700	19	2	11	7496	304	27	425	31

* As full enquiry as possible was made, and only such fatal cases are entered as there appeared to be reasonable ground for attributing to the results of vaccination.

The distribution of the lymph and the approximate number of cases of generalised vaccinia of which information was received is shown in the preceding Table (p. 141).

The climate in different parts varies considerably. Near the coast line it is humid, and during the cool season as hot in the day time as an English summer day in July or August. Some inland parts are of considerable elevation, from two thousand to four thousand and more feet above sea level; the sky in winter is generally clear, rain rarely falls, and though the sun's rays are strong the heat is tempered with a crisp atmosphere, while at night several degrees of frost are often registered. It is then possible that the freedom of the lower districts is attributable to a rapid reduction in virulence in the higher temperature. This is to some extent supported by the experience of one of us, that a portion of lymph which had been kept in a warmer place than the rest gave rise to no general eruptions.

We are not particularly concerned in this communication in the causes of generalised vaccinia, that is, the reason why the infection becomes generalised in some persons and remains a strictly local reaction in others. The condition is indicated by writers to be very uncommon. The natural conclusion from the paucity of cases in general is that generalised vaccinia is not due to any particular quality of the lymph used but to some other cause. In this instance, however, it is clear that some quality or character of the lymph itself was responsible for the consequences. It is assumed that the number given to a lymph issue is given for purposes of identification, and that all the lymph of the same number is derived from the same calf. That being so it appears that general vaccinia has ensued upon the inoculation of lymph of six different calves, which suggests that the peculiar quality was inherent in the strain of lymph, and not in the reaction of a particular calf. It is also likely that the effect was modified by atmospheric temperature, and that the small number of eruptive cases in the fourth up-country district after the use of 4698 was due to the exposure of that portion to a higher temperature while in possession of the vaccinator.

THE ACTION OF DYSENTERY BACILLI ON NITRITES AND NITRATES.

By W. J. LOGIE, M.B., CH.B.,
Carnegie Research Scholar.

*(From the Pathological Laboratory of the University and
Western Infirmary, Glasgow.)*

IN the course of work upon the inhibition of the cholera-red reaction in mixed cultures it was found that a marked difference exists between *B. dysenteriae* Shiga and *B. dysenteriae* Flexner in respect of their action on nitrites. This led to further investigations on the formation and destruction of nitrite by organisms of the dysentery group, which form the subject of the main part of this paper. In the first place, however, it has been necessary to examine the cultural methods hitherto employed for the identification and classification of organisms of the dysentery group.

It is convenient to separate the cultural and other characteristics into two divisions, (*a*) those which show that an organism belongs to the dysentery group, and (*b*) those which distinguish it as belonging to one or other of the various "types" or sub-divisions of the group. The general characteristics of dysentery organisms are as follows: they are short, plump, non-motile bacilli which give rise in the human subject to one form of the disease known clinically as dysentery. They may be isolated from the mucus occurring in the stools of dysentery patients or, after death, from the intestinal wall and mesenteric glands. From the organisms of the coli-typhoid group they are strikingly differentiated by the absence of flagella and consequently of motility. Their form is also plumper. In contrast to *B. coli* and *B. enteritidis* of Gaertner, they form no gas on any of the ordinary sugar media. None coagulate milk and none form acid from lactose, but almost all ferment

glucose. On agar they form thin film-like growths while on potato the growth which is at first whitish or transparent assumes ultimately a brownish tint. Gelatine is not liquified. On Endo-agar the colonies are pale. In staining reactions these organisms from cultures resemble *B. coli* being Gram-negative, but staining readily with the usual aniline dyes.

While dysentery organisms present characters which mark them off as a distinct group, they differ amongst themselves both in regard to serum-reactions and in their action on mannite and certain sugars, so that various subdivisions of the group have been proposed. The exact number of subdivisions to be adopted is not yet finally settled and so many variations exist that classification becomes difficult. Hiss (1904) recognises four types, of which the first ferments glucose alone; the second glucose and mannite; the third glucose, mannite and saccharose; and the fourth, glucose, mannite, saccharose, maltose and dextrine. To these, Shiga (1907) adds a fifth group which differs from type four of Hiss in that the culture media return to an alkaline reaction after five or six days and also on account of "wide differences" in agglutination reactions. It may be remarked that both Hiss and Shiga find that *B. dysenteriae* Flexner ferments saccharose, a result which is not confirmed by other observers. In this connection it should be noted that an organism which has been cultivated for some time on artificial media may acquire the power of fermenting substances which it could not at first attack.

Otto Lentz (1909) in his table gives the differential reactions of four types of which "Shiga," "Y" and "Strong," correspond to types I, II and III of Hiss respectively, while type "Flexner" differs from type IV of Hiss in not fermenting saccharose.

The following table shows the types of Shiga and Hiss, and their relationship to those of Lentz.

Name adopted by Hiss & Shiga	O. Lentz	Reaction given on media containing						Indol formation
		Glucose	Mannite	Saccharose	Maltose	Dextrine	Lactose	
Type I	Shiga	Acid	—	—	—	—	—	—
Type II	"Y"	Acid	Acid	—	—	—	—	+
Type III	Strong	Acid	Acid	Acid	—	—	—	+
Type IV	—	Acid	Acid	Acid	Acid	Acid	—	+
—	Flexner	Acid	Acid	—	Acid	—	—	+
Type V (Shiga)	—	Acid	Acid	Acid	Acid	Acid	—	+

The various mannite-fermenting strains seem to be more closely related to each other than to bacilli of the Shiga type. This is shown

particularly by serum-reactions, the serum of patients infected with bacilli of the Flexner or "Y" types agglutinating both *B. Flexner*¹ and the "Y" bacillus in equal dilutions, so that a differential diagnosis between these two organisms by means of the serum-reaction becomes impossible, whereas *B. Shiga* is not agglutinated by the sera of patients infected with these organisms. (Jürgens (1903), Lentz, Auché and Campana (1905).)

A further property which differentiates the Shiga strain is its much greater power of producing soluble toxins. This has led to a classification into a toxin producing type (giftiger Typus)—*B. Shiga*, and a relatively non-toxic group (giftarmen Typen)—the other dysentery bacilli.

While most writers agree that dysentery due to the Shiga type of bacillus is more severe and more fatal than the disease caused by the other members of the group, it is impossible to say from the clinical phenomena which type of bacillus is responsible for a particular case of dysentery. In this connection it may be mentioned that mixed infections occur and both Shiga and Flexner organisms may be obtained from the same case. No doubt this helps to confuse the clinical picture and make any differentiation of types due to different bacilli more difficult. Böse (1908) has even found amoebic and bacillary dysentery combined.

Of the organisms studied by the author three, which are all of the Shiga type, have been isolated by Dr Eyre from cases of asylum dysentery, while for six, the author is indebted to Professor Neisser of Frankfurt. One bacillus was isolated from a case of dysentery occurring in a Lascar, and the remaining four were obtained from Král of Prague.

Most of these had been cultivated on artificial media for some years, but the bacillus from the Lascar was examined when isolated and has preserved its properties unchanged during the two months it has been under observation.

With the exception of *B. Neisser* Ac.² all these organisms form acid readily from glucose. Some form acid (though not so readily as *B. coli*) in litmus-whey, but in lactose agar the reaction remains alkaline. This depends of course upon the fact that the litmus-whey is neutral to start with, while the agar medium besides being slightly alkaline con-

¹ The different types of *B. dysenteriae* hereinafter referred to are, for brevity's sake, named *B. Flexner*, *B. Shiga*, *B. Neisser*, etc.

² This organism was isolated from a severe sporadic case of dysentery. It is peculiar also in that peptone water cultures assume a distinct brown colour.

tains peptone from which alkali is liberated by the organism. The effect of the peptone is perhaps more strikingly shown by the fact that all the one per cent. mannite, maltose, and saccharose media, which had been fermented within 48 hours by *B. Flexner*, *B. Celli*, etc., had become alkaline by the end of 30 days. This return to an alkaline reaction has been noted also by other writers.

No gas is formed by these organisms on any of the usual media and milk is not coagulated.

Microscopically they appear as plump bacilli, of variable length, the tendency to the production of long forms being more marked in some strains than in others. *B. Flexner* for instance is on the whole a longer organism than *B. Jürgens*. In hanging drop preparations from young cultures the organisms are often seen joined end to end in pairs. Although Brownian movement is often marked, motility is absent.

The following table gives the reactions on one per cent. mannite, saccharose, maltose and dextrine agar¹, after 48 hours and also the results of testing for indol in 2 per cent. peptone water cultures after two weeks incubation at 37° C.

Organism	Mannite	Saccharose	Maltose	Dextrine	Litmus-whey	Indol
<i>B. Eyre</i> 7	—	—	—	—	—	—
„ 9	—	—	—	—	—	—
„ 10	—	—	—	—	—	—
<i>B. Neisser</i> Ac.	—	—	—	—	—	—
<i>B. Shiga</i> (Kral)	—	—	—	—	—	—
<i>B. from</i> Lascar	A	—	—	—	A	—
<i>B. Celli</i>	A	—	—	—	A faint	+
<i>B. Neisser</i> Mc.	A	—	—	—	A	—
<i>B. Jürgens</i>	A	—	A	A	—	+
<i>B. Flexner</i>	A	—	A	A	A	+
<i>B. Neisser</i> Lb.	A	—	A	A	A	—
„ Mb.	A	—	A	A	A	—
„ Nb.	A	—	A	A	A	—
„ Nc.	A	A	—	—	—	—

A = acid, — = no change.

B. Neisser Mc. ultimately (after about 10 days at 37° C.) forms acid from maltose, and *B. Neisser* Nc. after three or four days gives an acid reaction in litmus-whey. The *Neisser* organisms Lb., Mb., Mc., and Nb., all acidify litmus-whey more rapidly than *B. Flexner*, producing marked acidity in fourteen hours, whereas *B. Flexner* after that period gives only a very faint trace of acidity.

¹ 1 % peptone-water was used in place of bouillon in the preparation of the agar.

In the above table, seven groups may be distinguished. The most striking is that formed by the strains which do not ferment mannite, viz. *B. Shiga*, *B. Eyre* 7, 9, and 10, and *B. Neisser* Ac. The organisms which do ferment mannite fall into five groups: (1) the *Neisser* organisms Lb., Mb., and Nb. which all ferment mannite, maltose, and dextrine, but do not form indol; (2) *B. Flexner* which differs from these in forming indol; (3) *B. Jürgens*, which differs from *B. Flexner* in not forming acid in litmus-whey; (4) *B. Neisser* Mc., and the bacillus isolated from a Lascar, which ferment only mannite ("Y" type); (5) *B. Celli*, also "Y" type but differing from group (4) in that it produces indol; (6) *B. Neisser* Nc., which ferments mannite and saccharose (Strong type).

Formation and Destruction of Nitrite.

The medium used in these experiments was peptone water (peptone 1 %, sodium chloride .5 %) to which .00023 % sodium nitrite, or .000283 % sodium nitrate $\left(\frac{n}{30,000}\right)$ was added. The test used for nitrite was that with α -naphthylamin acetate and sulphanilic acid.

(1) Dissolve .5 gram. sulphanilic acid in 150 c.c. dilute acetic acid (sp. gr. 1040); (2) Boil .1 gram. naphthylamin acetate in 20 c.c. distilled water; filter and add filtrate to 180 c.c. dilute acetic acid (sp. gr. 1040). Mix these two solutions and keep protected from the air. At the time of using, the solution should be quite colourless.

A red colour, due to the formation of an "azo" dye, indicates the presence of nitrous acid or nitrites.

In studying the formation of nitrite two fallacies must be guarded against. In the first place, ordinary Witte's peptone as supplied for bacteriological purposes contains a trace of nitrite. In fresh 1 % solution the reaction may be very faint, but in 2 % solution it is more marked and in 5 % solution it is quite apparent. To obviate this source of error it is advisable to make all the peptone water for a single experiment at one time and to use an uninoculated tube of the medium (incubated along with the others) as a control. This "control tube" is useful also as a standard since in an experiment with plain peptone water organisms which destroy nitrite give a paler solution, while those which form nitrite give a stronger pink when tested with α -naphthylamin solution. It is thus possible by a single experiment to determine whether an organism forms or destroys nitrite.

The second source of error is the circumstance that nitrite and nitrate are present in the air. It has long been known for instance, that rain water contains traces of both, and the great difficulty of keeping the α -naphthylamin solution colourless serves to show how easily atmospheric nitrogen may complicate a reaction. In the course of these experiments it was found that under certain circumstances nitrite may be absorbed from the air in sufficient quantity to affect the result. The principal factors affecting the absorption are time, temperature, and amount of surface exposed.

Five c.c. of sterile water kept at 57° C. in a 3" \times $\frac{1}{2}$ " test tube, plugged in the usual way with cotton wool, give even after 19 hours a strong nitrite reaction. If kept at 37° C. a much longer time is required before the same depth of tint is obtained, only a very faint trace of nitrite being detectable after 24 hours. On the other hand if 5 c.c. of sterile water be placed in a 300 c.c. Erlenmeyer flask, plugged with cotton wool as usual, so that it forms a thin layer and exposes a large surface to the air quite a marked reaction is obtained even after 24 hours at 37° C.

When water is boiled for a short time in air, *e.g.* to sterilise it or in dissolving substances in it, no appreciable reaction is obtained, nor does sterilising for 1½ hours in a Koch's steriliser lead to the absorption of a detectable amount of nitrite.

That the nitrite reaction is due to absorption and not simply to concentration of nitrites already in solution, may be shown either by the use of distilled water or by taking 5 c.c. of fresh tap water and boiling till its bulk is reduced to below that of the water (originally 5 c.c.) which has been incubated. The simple concentration by boiling causes no nitrite reaction. Moreover, sealing the tube in a blow-pipe flame or in the case of incubation at 37° C. by means of paraffin wax, prevents the appearance of a reaction even after several weeks.

In the experiments in which the action of dysentery bacilli on nitrate and nitrite was tested all the tubes were sealed with paraffin wax before being placed in the incubator, and uninoculated tubes of the same medium were always incubated as controls along with the cultures. The following table gives the results obtained on testing with α -naphthylamin acetate and sulphanilic acid after 24 hours' incubation at 37° C.

From this it appears (1) that none of the organisms of Shiga type destroy nitrite; (2) that of the others, *B. Jürgens* and *B. Neisser* Nc. alone fail to destroy nitrite; (3) all except *B. Neisser* Ac. reduce nitrate. The number of types has now become eight since *B. Neisser* Ac. differs from the other Shiga strains in not reducing nitrate. *B. Jürgens* differs from *B. Flexner* in failing to destroy nitrite as

well as in giving no acid reaction in litmus-whey. As *B. Jürgens* forms both indol and nitrite, and fails to destroy nitrite it gives the cholera-red reaction.

Organism	Result of growth in medium containing	
	·000283 % nitrate	·00023 % nitrite
<i>B. Eyre</i> 7	Reduction to nitrite	Nitrite not destroyed.
„ 9	„ „	„ „
„ 10	„ „	„ „
<i>B. Neisser</i> Ac.	No nitrite	„ „
<i>B. Shiga</i>	Reduction to nitrite	„ „
<i>B. Celli</i>	„ „	Nitrite destroyed.
<i>B. Flexner</i>	„ „	„ „
<i>B. Neisser</i> Lb.	„ „	„ „
„ Mb.	„ „	„ „
„ Mc.	„ „	„ „
„ Nb.	„ „	„ „
<i>B. from Lascar</i>	„ „	„ „
<i>B. Neisser</i> Nc.	„ „	Nitrite not destroyed.
<i>B. Jürgens</i>	„ „	„ „

B. Flexner forms nitrite in 1 % peptone solution but destroys it within 24 hours. This may be shown by the following experiment.

200 c.c. of 1 % peptone water are inoculated with *B. Flexner* and placed at 37° C. Samples withdrawn by means of a sterile pipette are tested at intervals of an hour. During the first few hours no nitrite seems to be formed; but after about five hours the samples begin to show an increase in the amount of nitrite present and ultimately a strong nitrite reaction develops, the maximum being attained about 8—10 hours after inoculation. The amount of nitrite then rapidly diminishes and after about 18 hours the nitrite reaction has entirely disappeared.

Cultures of *B. Shiga* still give the nitrite reaction in full strength even after incubating for as long as four weeks.

The Effect of Oxygen upon Reduction.

It has been shown by Burri and Stutzer (1895), Weissenberg (1897) and others that free exposure to oxygen prevents certain organisms from reducing substances which under less aerobic conditions they may even reduce with ease. Cultivation of organisms in thin layers of medium or in a current of air or oxygen are methods which have been used. In the case of the dysentery organisms studied by the author the following method was adopted.

Five c.c. of nitrite peptone solution (peptone 1%, sodium chloride .5%, sodium nitrite .00023%) were placed in a conical Jena-glass flask of 300 c.c. capacity. The layer of fluid thus formed had a maximum depth of about 5 mm. and exposed a surface of about 45 sq. cm. The flask was fitted with a two hole indiarubber stopper through which passed two glass tubes one to within about $\frac{1}{2}$ " of the surface of the medium; the other but a short distance beyond the lower surface of this stopper. As they emerged from the stopper these tubes were bent at right angles and the horizontal portion was drawn out at one point to capillary dimensions in order to facilitate sealing. After the flask and its contained medium had been sterilised it was inoculated with the organism to be tested and the stopper tied in to prevent its being expelled by the expansion of the contained gas when the flask was placed in the incubator. The junction between the stopper and the neck of the flask and the points of emergence of the glass connecting tubes, were then luted with paraffin and the flask was connected in series with its fellows. As a rule from six to a dozen flasks were inoculated with different strains and treated at the same time, one flask being always kept sterile as a control. Sterilised rubber tubing was used to join up the series and from the last flask a longer piece of tubing led the escaping gases under water, thus obviating all risk of air re-entering the last flask, and at the same time affording a means of readily ascertaining whether gas was really passing. The importance of this may be understood when it is mentioned that the surface tension of a bubble of water such as often condenses in the capillary parts of the connecting tubes (especially during sterilisation) enables it to oppose such resistance to the passage of the gas that a flask will burst before the bubble yields. Gentle heating with a Bunsen-flame usually dispels such drops of water.

The oxygen was obtained from a cylinder and was passed through a series of wash-bottles, the first of which contained a saturated solution of urea acidified with hydrochloric acid, while the next two contained a strong solution of caustic soda. Two Drechsel wash bottles containing lead acetate and silver nitrate solution as used in purifying hydrogen were included on empirical grounds as it was found at the first attempt that the organisms did not grow, whereas on the second when these two wash-bottles were included growth was quite as good as that in $4" \times \frac{1}{2}"$ test tubes, sealed with paraffin in the manner already described. Samples of the gas issuing from the last flask were collected from time to time and tested with a glowing splinter of wood, or the glowing end of a piece of twine. When the sample of gas was able to re-ignite a glowing match, the gas was allowed to continue passing for some time and then the flow was stopped. The series was left from ten minutes to quarter of an hour to allow the pressure to become equalised, and then the flasks were sealed off one by one beginning with the flask nearest the oxygen cylinder. Similar flasks containing nitrate peptone solution (peptone 1%, sodium chloride .5%, sodium nitrate .000283%) were treated in the same way.

The results are included in the following table (p. 151).

In other experiments flasks were kept three or four days at 37° C., but no reduction had taken place even after that period.

It thus appears that oxygen completely inhibits the destruction both of nitrate and of nitrite. The growth in these flasks as judged by the degree

of turbidity produced was much the same as that of a similar series of cultures made in the same medium at the same time, but in $4'' \times \frac{1}{2}''$ test tubes which were sealed with paraffin. Lest the failure to destroy should be due to the death of the organisms, flasks inoculated with *B. Flexner*, *B. Neisser* Mc., *B. Neisser* Lb. and the bacillus isolated by the author were incubated at 37° C. for 48 hours in an atmosphere of oxygen, and agar slopes were then inoculated from them. Subcultures were obtained from all four flasks though no destruction of nitrite had taken place.

Organism	Result of testing for nitrite after 36 hours, at 37° C.		
	Nitrate series	Nitrite series	
<i>B. Eyre</i> 7	—	+	Same as control.
„ 9	—	+	„ „
„ 10	—	+	„ „
<i>B. Neisser</i> Ac.	—	+	„ „
<i>B. Shiga</i>	—	+	„ „
<i>B. Celli</i>	—	+	„ „
<i>B. Flexner</i>	—	+	„ „
<i>B. Neisser</i> Lb.	—	+	„ „
„ Mb.	—	+	„ „
„ Mc.	—	+	„ „
„ Nb.	—	+	„ „
„ Nc.	—	+	„ „
<i>B. Jürgens</i>	—	+	„ „
Control (uninoculated medium)	—	+	

In this connection it may be mentioned that Moore and Williams (1909) have studied the growth of *B. Shiga*, *B. Flexner*, and *B. Kruse*, in an atmosphere containing about 90 % oxygen. Following somewhat different methods they found that *B. Flexner* grew almost as well in 90 % oxygen as in air. The other two strains however showed wide variations in different experiments. The important point is that in the author's experiments growth as judged by turbidity was equal to that in the paraffin sealed tubes.

Effect of Anaerobic Conditions (Hydrogen).

With a view to testing whether anaerobic conditions might cause the non-reducing strains to attack the nitrite, the following experiments were performed.

A series of Jena-glass test tubes each containing 5 c.c. of nitrite peptone (peptone 1 %, sodium chloride .5 %, sodium nitrite .00023 %) were inoculated with the various strains of dysentery bacilli and hydrogen was passed through the tubes for a considerable time after the escaping gas burnt with a steady flame. They were

then sealed and incubated at 37° C., a control tube of uninoculated medium being treated in the same way. A similar series of tubes containing nitrate peptone solution was also treated in the same fashion.

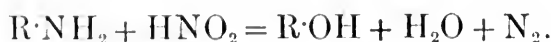
The following table gives the results.

Organism	Result of testing for nitrite after 36 hours at 37° C.				
		Nitrate series			Nitrite series
<i>B. Flexner</i> (Kral)	+	Like nitrite series control			—
<i>B. Celli</i> ,,	+	,,	,,	,,	—
<i>B. Jürgens</i> ,,	+	,,	,,	,,	Same as control.
<i>B. Shiga</i> ,,	+	,,	,,	,,	,, ,,
<i>B. Eyre</i> 7	+	,,	,,	,,	,, ,,
<i>B. Neisser</i> Ac.	—	Like nitrate series control			,, ,,
,, Lb.	+	Like nitrite series control			—
,, Mb.	+	,,	,,	,,	—
,, Mc.	+	,,	,,	,,	—
,, Nb.	+	,,	,,	,,	—
,, Nc.	+	,,	,,	,,	Same as control.
Control tube of uninocu- lated medium treated in the same way	—	—			+

It thus appears that under anaerobic conditions both the reducing and the non-reducing strains behave exactly as in the paraffin sealed 4" × ½" test tubes, the non-reducing strains still failing to destroy nitrite though they reduce nitrate as usual. In some experiments the tubes were allowed to stand for several days, in one case for a week, before testing, but no reduction was effected even in that period.

The Effect of Glucose on Nitrite Destruction.

Although as has been already shown, *B. Shiga*, *B. Jürgens*, and *B. Eyre* 7, 9, and 10, fail to destroy nitrite which has been added to ordinary 1 % peptone solution, the further addition of 1 % glucose to the medium enables them to do so quite as thoroughly as *B. Flexner* or *B. Celli*. Since all of these organisms form acid from glucose, this resulting destruction is no doubt due, as Grimbart and Bagros suggest, to the formation of acid which displaces the nitrous acid from its compound and so enables it to react with amide nitrogen, according to the equation:



The mechanism of this reaction will be dealt with in a future communication.

Effect of Oxygen on Nitrite Destruction in Glucose-containing Media.

To test the effect of oxygen upon nitrite destruction in glucose-containing media the following experiment was carried out.

Six flasks fitted with indiarubber stoppers and glass connecting tubes were prepared as before and in each was placed 4.5 c.c. nitrite peptone solution. After sterilisation, .5 c.c. of a sterile 20 % solution of glucose was added to each of three, and .5 c.c. of sterile water to each of the other three. Of each set of three, one flask was inoculated with *B. Shiga*, one with *B. Flexner*, and one was kept sterile as a control. After inoculation the flasks were connected in series and oxygen passed as before, the flasks being finally sealed and placed in the incubator at 37° C. After 80 hours each was tested for nitrite.

The following table gives the results.

Organism	The result of testing for nitrite the flask containing	
	Glucose	No glucose
<i>B. Flexner</i>	Faint colour (destruction)	Marked colour (like control).
<i>B. Shiga</i>	" "	" "
Medium control treated } in the same way }	Marked colour	" "

It is thus apparent that marked destruction had occurred with both organisms in the presence of glucose although not to quite so marked an extent as is the case in sealed test-tubes. This destruction on the part of *B. Flexner* contrasts with the total absence of destruction in the corresponding flask inoculated with *B. Flexner*, and treated in precisely the same way, but containing no glucose. With both organisms abundant growth had occurred in the glucose medium as was shown both by turbidity and on microscopic examination. To test whether the conditions of growth had affected the vitality of the organisms sub-cultures were made on agar and growth resulted except from the glucose peptone culture of *B. Shiga*. It is most likely that the acid produced had caused the death of this organism, which is, in general, more delicate than *B. Flexner*. At any rate, growth was more abundant in this flask than in any of the others and the death of the organism cannot therefore be due to inhibition by the oxygen.

SUMMARY AND CONCLUSIONS.

(1) Dysentery strains from various sources have been examined with respect to their power of reducing nitrates to nitrites and of further destroying nitrites. All the strains with one exception (*B. Neisser* Ac.) reduced nitrates to nitrites. None of the strains which fail to ferment mannite (Shiga type) destroyed nitrites. Those dysentery strains which

form acid from mannite differ amongst themselves in regard to their action on sugars and also in their effect on nitrites. Of the eight mannite-fermenting strains examined, two (*B. dysenteriae* Jürgens and *B. Neisser* Nc.) failed to destroy nitrites.

(2) *B. dysenteriae* Jürgens, although closely related to *B. dysenteriae* Flexner, differs from it both in its action on litmus-whey and in failing to destroy nitrite. It must therefore be considered a different strain. *B. dysenteriae* Jürgens is remarkable as being the only one of the strains examined which forms indol and at the same time fails to destroy nitrites. It is consequently the only one which gives the cholera-red reaction.

(3) The addition of glucose (*i.e.* of a carbohydrate from which acid can be formed) enables Shiga strains to destroy nitrite.

(4) In the presence of an abundant supply of oxygen all the strains fail to destroy nitrites and nitrates.

(5) In glucose-containing media the inhibitory effect of oxygen is less marked.

(6) Under anaerobic conditions Shiga strains and *B. dysenteriae* Jürgens still fail to destroy nitrites.

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THE ETIOLOGY OF TYPHUS FEVER.

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TYPHUS FEVER is a disease which modern sanitary reform has banished from the midst of progressive communities. As a result few opportunities are now afforded for investigating the disease by the bacteriological methods of to-day. Sporadic cases and small epidemics occur from time to time in the few insanitary districts that remain in Belfast. Dr Ernest H. Milligan and I made some observations of a bacteriological nature on this disease which we communicated to the Ulster Medical Society in 1908. Since that time I have continued the investigations when any opportunity occurred and in this paper are recorded the results which have been obtained. Recently some new light has been thrown on the etiology of typhus fever, so the present time appeared to me to be opportune to publish our own observations, to analyse the findings of other observers and to discover what is truly established with regard to this subject.

I have divided the paper into sections in each of which I first relate the observations made in Belfast, then those of other investigators and finally I state the conclusions which I think can be fairly drawn in the present state of our knowledge.

I.

The microscopic examination of the blood.

Blood films were made from 12 patients at various stages of the fever. These were at once fixed in absolute alcohol and afterwards coloured by Leishman and Giemsa's dyes, methylene blue, dilute carbol-fuchsin and thionin blue. The stains were allowed to act from 5 minutes to 24 hours. A careful search for protozoon parasites was made but only on one occasion was a body found which bore any resemblance to such organisms. This structure, which was ovate in shape, measured about $6\ \mu$ in length and $1.5\ \mu$ in breadth and contained two little round deep red-stained dots (Giemsa's stain was used), one being centrally situated the other at one of the poles. It was very similar in appearance to Fig. 6 of the bodies described by Krompecher, Goldzieher and Augyán (1909).

The most striking feature of the blood films was the great number of large mononuclear cells and the absence of eosinophile cells. I made a differential count of several thousand white cells in films taken at different stages of the disease in 12 different patients. The following is a brief summary of my findings. In no case was an estimation of the total number of white cells present determined but there was evidently a leucocytosis in most of the cases.

Polymorphonuclear leucocytes. During the febrile period the relative proportion of polymorphonuclear cells varied from 50 to 80 %. On an average 70 % of the cells belonged to this class.

Large mononuclear cells of Ehrlich. In this group we include hyaline and transitional cells. A relative increase of these cells was one of the most striking features of the films. The nuclei of these cells were round, oval or reniform and the cytoplasm was abundant. Vacuoles were sometimes seen in the cytoplasm and on one occasion in the nucleus itself.

Occasionally large cells were seen with two nuclei, giving one the impression that the cell was in the act of division. The large mononuclear cells increased in relative proportion as the disease advanced, and when the temperature dropped to normal they formed on an average

25 % of the leucocytes. The increase of mononuclear cells persisted during convalescence; patients whose temperature had been normal for four weeks had about 20 % of their leucocytes of this type.

Lymphocytes. The lymphocytes large and small were relatively reduced, their place apparently being taken by the large mononuclear cells. On an average the combined large and small lymphocytes amounted to 10 % of the leucocytes.

Eosinophiles. During the acute stage of the disease these were absent or present in very scanty numbers. In 3755 leucocytes counted during the febrile period only 8 were eosinophiles, a proportion of .2 %. With the defervescence of the fever their numbers rose and soon they regained their normal proportion. In two cases we observed a subsequent eosinophilia during convalescence, the eosinophile cells forming in these cases 12.8 % and 16.5 % respectively of the leucocytes.

Myelocytes. An occasional myelocyte was found in some of the films.

Having thus given the results of my own examination I shall now refer to those recorded by other observers.

Mott and Blore (1883) in examining drops of fresh blood taken from 12 cases of Typhus Fever saw dumb-bell shaped bodies which they regarded as micrococci undergoing division. Single cocci $.5\mu$ in diameter were also seen. Cultures were not made. They noted that the white blood corpuscles were increased.

Thoinot and Calmette (1891) found a leucocytosis present in the blood of four Typhus cases examined. In the blood withdrawn by splenic puncture in four cases and in that obtained from the finger in a fifth case they saw little refractile bodies 1μ to 2μ in diameter provided with a short cilium and moving with great rapidity between the red cells. When the same drop of blood was examined 12 to 24 hours later the little bodies were not visible but instead refractile threads 10μ to 30μ in length were seen displaying a serpentlike motion amid the blood cells. These filaments had at one end a round or oval swelling equal to $\frac{1}{3}$ or $\frac{1}{4}$ the diameter of an erythrocyte. Thoinot and Calmette were unable to obtain any cultures. They recognised that bodies similar to those they described had been observed in other diseases so that they were unable to decide whether these structures represented stages in the breaking down of blood corpuscles or whether they were specific elements.

Soon after Lewaschew (1892) described structures in fresh blood which very closely resembled blood platelets but differed in having a smaller volume, a more regular outline, greater and more spontaneous

motility and also in the possession of a thread-like process. In short the figures and descriptions of the bodies of Thoinot and Calmette and those of Lewaschew are very similar. Lewaschew obtained cultures of cocci which varied from 2μ to 5μ in diameter and which had often long cilia. He believed that these cocci represented a stage in the life-cycle of the bodies which he saw in the fresh blood as is evident from the following quotation: "although one usually in the investigation of Typhus blood finds three forms of micro-organisms—cocci, cocci with threads attached, and free threads (spirochaetae), it is very probable that these represent only different forms of one micro-organism."

Benjasch (1899) and Stanichevskaja (1905) were able to confirm the discovery of Lewaschew, whilst Weinschal (1892) in 10 cases obtained entirely negative results both as regards microscopic and cultural examination of the blood.

Gotschlich (1903) described in the blood of Typhus cases parasites which in his opinion stood nearly related to *Piroplasma (Babesia) bovis*. His descriptions of the bodies are as follows: (1) Endoglobular pear-shaped parasites varying in diameter from 1μ to 4μ . These were observed in six cases. (2) Flagellated bodies like spermatozoa consisting of an oval body 1.5μ in its long axis and provided with a long winding flagellum. These bodies were actively motile and were very similar in appearance to those described by Thoinot and Calmette and Lewaschew. (3) Cysts of an oval or round form and about the size of erythrocytes. These contained 3—6 intensely staining round bodies at the edge and were regarded by Gotschlich as sporulation forms. They were found in only a single case. Kireef (1905) and Horiuchi (1908) were unable to confirm Gotschlich's observations, as in their hands microscopic and cultural examination of Typhus blood gave negative results.

Love (1905) concluded (1) that Typhus Fever is always accompanied by a leucocytosis. The average number of leucocytes in a series of 26 cases was found to be 24,000, the numbers ranging between a minimum of 8000 and a maximum of 54,000 per cubic mm.; (2) that the character of the leucocytosis is practically the same in all cases and corresponds with that found in certain of the other exanthemata, e.g. scarlet fever, inasmuch as it results mainly in an increase of polymorphonuclear cells; (3) that in the blood of fatal cases there are no eosinophile cells whilst in non-fatal cases these corpuscles are always present; (4) in non-fatal cases there is occasionally a slight relative increase in the large mononuclear elements; (5) that the red corpuscles are usually increased in numbers.

Love contrasted the leucocytosis of Typhus Fever with the leucopenia of Typhoid Fever and in his opinion a blood examination would be sufficient to distinguish the two diseases "though," he admitted, "there is some apparent similarity in the manner in which the large mononuclear and eosinophile cells behave." The character of the blood in Typhus suggested to him that the disease was due to a diplococcus since it was very similar to that found in Pneumonia and Rheumatic Fever. Love was unable to confirm Gotschlich's opinion as to the presence of protozoa in the red blood corpuscles and although he met with bodies somewhat similar to those described by Gotschlich he regarded them as portions of the corpuscles that had undergone degenerative changes.

Slatinéano and Galesesco (1906) made a cytological examination of the blood of 16 cases. They concluded their paper with these words: "In Typhus Fever there is a polymorphonuclear leucocytosis and the variations in this are explained by the fact that in the course of the disease there are produced a series of secondary infections. But what strikes the attention is the enormous and constant increase of the mononuclears which is attained at the end of the disease. They may amount to 45% of the leucocytes. It may be supposed that this increase of mononuclears represents the reaction of the body against the unknown parasite."

On examining a drop of fresh blood they found in most cases a little dumb-bell shaped corpuscle 2—3 μ in length and having the poles more refractile than the central part. It exhibited slight movements of oscillation but not of translation. This little corpuscle was sometimes phagocytosed by the mononuclears and lay in a vacuole within them. Vital staining by neutral red and methylene blue showed two polar points more deeply stained than the central part. They never found the body in preparations stained in the usual way.

Lucksch (1907) examined the blood of 21 cases and found with but few exceptions a leucocytosis present. The eosinophiles were absent and the mononuclear cells were increased. No micro-organisms were found in the blood by cultural or microscopic examination. Krompecher, Goldzieher and Augyán (1909) in blood films made from 48 cases of Typhus Fever and stained by Giemsa's method saw structures which in their morphological and staining characters as well as in their position inside the red blood corpuscles resembled at one time malarial parasites at another piroplasmata. They considered the possibility of the bodies being degenerated portions of corpuscles but rejected this interpretation and concluded that they were protozoa.

SUMMARY OF SECTION I.

From these various observations we are justified in drawing the following conclusions:

(1) That in Typhus Fever there is generally a leucocytosis (Mott and Blore, Love, Slatinéano and Galesesco, Lucksch).

(2) That the polymorphonuclear leucocytes are increased but that the most striking feature is the increase of the large mononuclear cells and the absence of eosinophiles (Slatinéano and Galesesco, Lucksch, Wilson and Milligan).

(3) That a post-febrile eosinophilia is sometimes observed (Wilson and Milligan).

(4) That it must be left an open question whether the flagellated bodies described by Thoinot and Calmette and Lewaschew are parasites or breaking-down cells though on the whole the evidence points to the latter conclusion.

(5) That the dumb-bell shaped bodies described by Mott and Blore and by Slatinéano and Galesesco in my opinion represent micrococci in the act of division.

(6) That the protozoon nature of the bodies described by Gotschlich, Krompecher, Goldzieher and Augyán and myself has not been established.

II.

**The bacteriological examination of the blood,
cerebro-spinal fluid, organs, &c.**

Our routine procedure was to withdraw aseptically from the median basilic vein 5—10 c.c. of blood and to introduce it into a flask containing 100 c.c. of bouillon. The flask was then incubated at 37° C. for 24—48 hours and subcultures made on solid media. Occasionally we used glucose bouillon and several times the blood was received in melted agar at a temperature of 43° C. For subculturing from the flasks the solid media used were agar, ascitic-agar, glucose-ascitic-agar. In a few cases the blood was mixed with an equal quantity of 1% potassium citrate solution and then incubated at room and body temperature. It was only when we introduced the blood into bouillon that we succeeded in obtaining cultures.

Thirty-three cases were examined, in 18 instances with negative results whilst in 15 cultures of diplococci were obtained. In most cases the examination was made about the end of the first or early in the second week of the illness.

The characters of the diplococci isolated.

Morphology. Small gram-positive diplococci: occasionally short chains consisting of four or six individuals were seen. Growth on agar and gelatin—greyish film, bluish by transmitted light. No formation of pigment. Growth rather like a culture of the *B. typhosus*, quite unlike a culture of *Staphylococcus aureus* or *albus*. In about half the cases liquefaction of the gelatin commenced about the tenth day whilst in the others none was evident even after six weeks' cultivation. In bouillon a uniform turbidity was produced. In one case an ovoid gram-positive diplococcus or streptobacillus was isolated. This differed from the others in showing a more delicate film of growth and in the rapidity with which it fermented raffinose and clotted milk. The fermentative activity of these cocci was investigated by growing them in litmus broth containing 1% of certain sugars, alcohols, glucosides, and aldehydes. The results obtained with two strains of the diplococci and with the streptobacillus are seen in Table I.

TABLE I.

	Glucose	Laevulose	Galactose	Maltose	Saccharose	Lactose	Glycerine	Mannite	Dextrin	Sorbit	Rhamnose	Xylose
Diplococcus No. 1	+	+	+	+	+	+	+	+	+	-	-	-
„ „ 2	+	+	+	+	+	+	+	-	+	-	-	-
Streptobacillus	+	+	+	+	+	+	-	-	+	-	-	-
	Raffinose	Arabinose	Adonite	Dulcite	Salicin	Amygdalin	Inulin	Litmus milk	Gelatin			
Diplococcus No. 1	-	-	-	-	-	-	-	clot	no liquefaction			
„ „ 2	-	-	-	-	-	-	-	clot	slow „			
Streptobacillus	+	-	-	-	-	-	-	clot	no „			

The sign + denotes formation of acid; - indicates no change of reaction.

It will be seen that the two strains of diplococci differ from each other in that the one ferments mannite and does not liquefy gelatin whilst the other ferments mannite but liquefies gelatin slowly.

From different members of the same family we have on several occasions obtained cultures of diplococci which in all their biological characters were identical.

Pathogenicity. Mice and guinea-pigs inoculated subcutaneously suffered no ill effects.

On only one occasion had we an opportunity to make a post-mortem examination. In this case smears from the spleen on agar gave no growth but a little piece of spleen added to glucose-ascitic bouillon and incubated afforded a growth of cocci which in all their characters resembled diplococcus No. 2.

Lumbar puncture was performed in one case and the cerebro-spinal fluid was found to be sterile.

Opsonins and Agglutinins.

The opsonic index of the serum of four cases was tested from time to time with reference to diplococcus No. 1. The index was found to vary from .5 to 1.9, rising as convalescence approached. Though we do not lay much stress on our results still they support the view that the cocci had actually been obtained from the patients' blood and were not mere contaminations from the skin. Agglutination experiments afforded strong confirmatory evidence of this view.

In testing for agglutinins emulsions in normal salt solution of a 24 hours' agar culture of diplococcus No. 1 and diplococcus No. 2 were used. Such emulsions had no tendency to spontaneous clump formation. Diplococcus No. 2 was agglutinated in slightly higher dilutions than diplococcus No. 1 both by normal and by typhus serum. The results were recorded at the end of two hours at room temperature.

We tested the action of the blood serum of 13 different cases of Typhus Fever and found that it had from 5 to 20 times the agglutinative effect of normal serum. In all cases we made control tests with the blood of healthy men. An example of an agglutination experiment is given in Table II.

In the case of six healthy adults a dilution of 1 in 20 was the limit at which agglutination occurred. In a few cases of typhoid fever and cerebro-spinal fever we got agglutination in dilutions of 1 in 100. This result certainly impugned the value of the reaction but we must remember that in these diseases heterologous agglutination is a frequent phenomenon.

TABLE II.

Agglutination of Diplococcus No. 1 isolated from the blood of case No. 1.

	Dilutions employed							
	1 in 20	50	100	200	300	400	500	600
Typhus patient's serum No. 1	+++	+++	+++	+++	+	+	+	-
„ „ „ No. 2	+++	+++	+++	++	+	-	-	-
„ „ „ No. 3	+++	+++	+++	+	+	-	-	-
Controls:								
Appendicitis case ...	+	-	-	-	-	-	-	-
Normal blood serum No. 1	+	-	-	-	-	-	-	-
„ „ „ No. 2	+	-	-	-	-	-	-	-

In this and the following tables +++ indicates marked, ++ moderate, + slight agglutination; - absence of agglutination.

These agglutinins could be removed by saturating the serum with the coccus. One drop of the serum was taken and 19 drops of normal salt solution added to it. To this several loopfuls of the growth from an agar culture of the coccus were added. After four hours the mixture was centrifugalised and it was then found that the supernatant serum had lost its agglutinative action on the coccus. In Table III details of such an experiment are given.

TABLE III.

	40	60	100	200	300	400
Serum before saturation	+++	+++	+++	+++	+	+
„ after „	-	-	-	-	-	-

An experiment was devised to determine whether the agglutinins acting on diplococcus No. 1 and on diplococcus No. 2 were the same or distinct. Such an experiment is shown in Table IV. The serum was that of a patient in the ninth day of the disease.

TABLE IV.

Titre of the original serum.		Dilutions employed						
		40	60	100	200	300	400	500
Diplococcus No. 1		+++	+++	++	+	+	-	-
„ „ 2		+++	+++	+++	+++	++	+	-
Titre of serum which had been saturated with diplococcus No. 1 for 16 hours at room temp.								
Diplococcus No. 1		-	-	-	-	-	-	-
„ „ 2		+++	+++	++	+			
Titre of serum which had been saturated with diplococcus No. 2 for 16 hours at room temp.								
Diplococcus No. 1		+	-	-	-	-	-	-
„ „ 2		-	-	-	-	-	-	-

This experiment shows that saturation with diplococcus No. 2 removes all its own agglutinins and most of the agglutinins that act on No. 1. Saturation with diplococcus No. 1 removes all its own agglutinins but only half of the agglutinins that act on diplococcus No. 2.

In a paper published in 1893 A. Gouget gave a summary of the bacteriological investigations that had been made up to that date with regard to Typhus Fever. We quote the following passage from that paper: "In 1881 Brautlecht isolated from the urine of several Typhus Fever patients a microbe which he considered characteristic but which has never since been found. The same objection applies to the micrococcus observed by Hallier in the blood and sought for in vain by Rosenstein as well as to the bacillus described by Moreau and Cochez. Mosler found the blood of several Typhus cases taken during life sterile and like Obermeier he had negative results when he inoculated animals with such blood. Zülzer in the course of his animal-inoculation experiments obtained one positive result but doubt is cast on its specific nature by Eichhorst. Obermeier obtained no result on inserting the blood of a Typhus case under the epidermis of a healthy man."

Hlava (1888) obtained cultures of gram-positive streptobacilli from the blood. On agar, serum and in bouillon good growth occurred whilst there was no growth on gelatin and on potato. The cultures were not pathogenic for the usual laboratory animals but two young pigs when inoculated presented febrile symptoms lasting a fortnight and the one which had been inoculated in the lung presented reddish blotches on its skin. Hlava also obtained cultures of staphylococci and pneumobacilli.

We have already referred to the fact that Mott and Blore (1883) saw in drops of fresh blood bodies that they regarded as diplococci and that Lewaschew (1892) obtained cultures of micrococci. Calmette (1893) obtained from the blood, sputum and urine of six cases of Typhus Fever cultures in acid media of a fungus which belonged to the class of Ascomycetes or Ustilagineae; he believed that the bodies previously seen by him and Thoinot in the microscopic examination of the blood represented a stage in the life-history of this organism.

Dubief and Bruhl (1894) examined bacteriologically nine cases of Typhus Fever and on six of these they made a post-mortem examination. Cultures were difficult to obtain from the blood though diplococci could be seen on microscopic examination, but from the sputum and from the pneumonic patches in the lungs cultures were readily obtained. The diplococcus named by its discoverers *Diplococcus exanthematicus* grew well on the ordinary media and liquefied gelatin. On agar the

growth appeared as a greyish streak at the end of 24 hours, at the end of 48 hours it became yellowish but finally assumed its former colour. Animal inoculation did not yield satisfactory results.

Curtis and Combemale (1893) were unable to obtain cultures from the blood of patients during life but from the spleen and brain of three fatal cases they obtained cultures of diplococci which very closely resembled those described by Dubief and Bruhl. Weinschal (1892) in 10 cases, Fuchs (1896) in 7 cases, Spillmann (1896) in 5 cases, McWeeney (1898) in 2 cases failed to obtain any cultures of micro-organisms.

Balfour and Porter (1899) from drops of blood obtained cultures of gram-positive diplococci in 36 out of 43 cases examined. Control examination of the blood of healthy individuals yielded negative results but a similar organism was obtained in 40 out of 46 cases of Typhoid Fever. They described the growth on agar as similar in appearance to a streak of white cement, copious and rapid in its spread. Liquefaction of the gelatin began about the fourth day.

Stanichevskaja (1906) examined by cultural methods the blood of 19 cases of Typhus Fever. Cultures were obtained only when large quantities (4—5 c.c.) of blood were withdrawn into 100 c.c. of broth. In 11 cases the flasks remained sterile, whilst in 6 cultures of diplococci were obtained which from the description were probably identical with those isolated in Belfast. Of the 2 remaining cases one gave a growth of a diplobacillus and the other of a bacterium.

Kireef (1905) in 12 cases examined obtained negative results in 9 and in the remaining 3 where cultures of streptococci were obtained he attributed the growth to a contamination.

Galesesco and Slatinéano (1906) found the blood in 18 out of 24 cases sterile. On four occasions a gram-positive diplococcus and on six a gram-negative non-motile bacillus was discovered. This same bacillus was found in the cerebro-spinal fluid of eight out of 24 cases examined. In five cases an autopsy was made; the heart's blood of four gave a culture of gram-positive diplobacilli and of one the gram-negative bacillus with a streptococcus. The cerebro-spinal fluid yielded in four cases out of the five the gram-negative bacillus accompanied by pneumococci. The gram-negative bacillus possessed the following characters. It was non-motile, reddened the Drigalski medium, fermented lactose with formation of acid but no gas, coagulated milk in 48 hours, formed indol in 24 hours and did not liquefy gelatin. It was slowly agglutinated in dilutions of 1:50 and 1:100 by the blood serum of Typhus patients.

Galesesco and Slatinéano regarded these micro-organisms as secondary invaders and believed that the increase of the large mononuclear cells in the blood pointed to the pathogenic agent being a protozoon.

Krompecher, Goldzieher and Augyán (1909) found in the blood streptococci, staphylococci and pneumobacilli which they regarded as secondary invaders and which probably aggravated the clinical condition.

Rabinowitch (1909) in sections by special methods of staining demonstrated gram-positive bacilli and also obtained cultures of the same.

SUMMARY OF SECTION II.

Little weight can be attached to the result of blood-culture experiments performed before 1900 as prior to that date in most cases only drops of blood were taken or the results were based on the examination of too small a number of cases. The results of modern investigators (*e.g.* Stanichevskaja, Galesesco and Slatinéano, Wilson and Milligan) show that gram-positive diplococci can in a large proportion of cases be cultivated from the blood. Our agglutination experiments tend to show that such organisms were actually infecting the persons from whom they were isolated. Whether the diplococci described by various observers should be regarded as identical is very doubtful. The diplococci of Dubief and Bruhl formed pigment, and those of Balfour and Porter produced a growth "resembling a streak of cement," descriptions which suggest that these observers were dealing with cultures of the *Staphylococcus aureus* and *Staphylococcus albus* respectively. We believe that the organisms described by Stanichevskaja and by ourselves are identical. In our cases the cultures on agar were semi-transparent and there was a complete absence of the pigment formation and opaqueness characteristic of cultures of staphylococci. Whether these diplococci are to be regarded as the causative organisms or merely as secondary invaders must be left undecided at present: recent investigation supports the second hypothesis. With this we shall now deal in Section III.

III.

The Virus.

Mochutkovski (1900) after seven unsuccessful attempts at last succeeded in infecting himself by inoculation with the blood of a Typhus Fever case. The incubation period was 17 days.

Yersin and Vassal (1908) succeeded in conveying Typhus Fever to two natives of Indo-China by subcutaneous injection of .5 c.c. of blood taken from patients in the second and fifth day of the disease. The incubation period in the first case lasted 14 and in the second 21 days. Yersin and Vassal could discover no signs of the virus in the blood but like Galesesco and Slatinéano and ourselves they found towards convalescence a characteristic increase of the large mononuclear cells.

Nicolle (1909) transmitted Typhus Fever from a patient to a chimpanzee by inoculating the latter with 1 c.c. of the patient's blood. From the chimpanzee the infection was conveyed to a monkey (*Macacus sinicus*) although direct inoculating of such an animal with the blood of an infected man gave negative results. Nicolle's experiments proved the presence of the virus in the blood of man on the day on which the eruption first appeared and in the blood of the chimpanzee two days before its appearance.

Anderson and Goldberger (1910) conveyed to two species of monkeys, *Macacus rhesus* and *Cebus capuchinus*, a characteristic fever by inoculation with the blood of Typhus Fever cases. After an incubation period of 5—11 days the fever continued for 13 days and in its onset, course, duration and critical defervescence presented a striking resemblance to the fever curve of this disease in man. The eruption was absent. The human blood with which they inoculated gave no visible growth on ordinary culture media. Passage experiments supported the view that they were dealing with a living virus capable of multiplication. The virus was apparently too large to pass through a Berkefeld filter since the authors found that diluted defibrinated human blood after filtration when inoculated into monkeys gave no febrile reaction or other manifestations of illness.

Ricketts and Wilder (1910) in this and other points independently obtained results similar to those of Anderson and Goldberger. These experiments of Anderson and Goldberger strongly support the view that the micro-organisms described by various observers should be regarded as only secondary invaders and that the virus cannot be grown on the ordinary media. We may note however that we have seen what appeared to be diplococci in the blood of some of the cases from which we were unable to obtain cultures.

[illegible]

We may add that the agglutinins which acted on the diplococci and which acted on the *B. typhosus* and on the *Bacillus U.* were proved by saturation experiments to be distinct.

Horiuchi (1908), in a fever clinically resembling Typhus Fever, obtained from the stools and in 3 cases from the urine of 40 cases examined a bacillus which we found to be culturally identical with the *Bacillus U.* Horiuchi believed that the fever in question was due to his bacillus and the fact that some of the cases agglutinated the *Bacillus typhosus* also he attributed to the presence of group agglutinins. The bacillus was never obtained from the blood.

The fact that intestinal organisms should be found by different observers to be agglutinated by the blood serum of Typhus Fever cases is interesting but what its significance is cannot be at present determined. In Section V we shall refer to this matter in greater detail.

V.

Differentiation of Typhus from Typhoid Fever.

The fact that it was not until the fourth and fifth decades of the nineteenth century that these diseases were recognised as two separate entities shows how closely akin the older physicians imagined them to be. Those who have much experience of Typhus Fever know that there are cases in which it is impossible to make a clinical distinction. Ker refers to such cases when he states "but even if we fail to make the distinction clinically we have always the Widal test at our disposal." I shall presently show that the Widal test affords but little assistance.

Many writers separate Typhus Fever from Typhoid Fever and classify it with the acute exanthemata (Small Pox, Scarlet Fever, Measles) which it resembles in its infectivity, onset and rash; on the other hand it differs entirely from these in the fact that it is always associated with unhygienic conditions whilst the other diseases named have never been shown to be influenced as to their incidence by good or bad sanitation.

We have already referred to the fact that in Typhoid Fever a leucopenia and in Typhus Fever a leucocytosis is the rule. However in many respects there is a resemblance in the blood picture presented by Typhus and Typhoid Fever. Thayer (1902) states that the three main changes in the blood in Typhoid Fever are (1) a progressive diminution

in the percentage of the polymorphonuclears, (2) a progressive increase in the percentage of mononuclear forms, the increase being mainly in the large mononuclear varieties, (3) a constantly small percentage of eosinophiles. We have noted that all these changes occur in the blood of Typhus cases. There is no doubt that in Typhoid and Typhus Fever the blood is characterised by an increased proportion of large mononuclear cells. We may attribute this reaction to the view that the causative organism is a protozoon; or on the other hand may it not be due to the fact that in both diseases there is an infection with organisms of the coli-typhoid group? We believe that our experiments and those of Horiuchi prove that in Typhus Fever such an infection (secondary though it probably is) does occur. Such infecting organisms in Typhus however do not enter the blood stream though they may be found in specimens of urine taken with every aseptic precaution. They probably reach the urine through the lymphatic system. We may here recall Coleman and Buxton's (1909) view with regard to the pathogenesis of Typhoid Fever. These observers believe that in this disease the atrium of infection is the lymphatic structures of the intestinal wall whence the bacilli invade the general lymphatic system and spleen and from these systems the blood is only secondarily invaded.

As regards the value of the Widal test in assisting in the differential diagnosis the following is our experience. The blood serum of 35 cases of Typhus Fever was tested in reference to its agglutination of *B. typhosus*. In 19 instances the reaction was positive; in 16 it was negative where a 1 in 50 dilution of the serum was used. In several cases higher dilutions were employed: in many of these good clumping was obtained in 1 hour in a 1 in 100, in several in a 1 in 200 and in one in a 1 in 300 dilution. On several occasions different strains of typhoid bacilli were employed and almost invariably our results were controlled by the examination of non-typhus blood. I may add that the patients from whom the blood was taken were undoubtedly suffering from Typhus Fever. The clinical picture was typical and in nearly all cases many members of the same family were attacked. Moreover the blood was examined by cultural methods in 33 cases and the flasks either remained sterile or there was a growth of diplococci but never of bacilli. We may conclude that the Widal test for the purposes of diagnosis is of little value in this connection. Patterson (1908), from observations in Lanarkshire cases, independently reached the same conclusion.

Patterson made 38 Widal examinations of the blood of 10 cases of Typhus Fever, employing seven different strains of typhoid bacilli. In nine cases a positive and in one a negative reaction was obtained. The dilutions of the serum employed ranged from 1:50 to 1:1000 and in four cases a positive result was obtained at some stage of the disease even with the latter dilution. The faeces, urine and blood were culturally examined for the *B. typhosus* but with negative results. Patterson found that the reaction was obtained as early as the ninth day of the illness, was most marked about the fourth week and gradually passed off about the sixth week.

Patterson's results and ours differ from those recorded by most writers on this question as is evident from the following quotations:— "After trying the Widal reaction in nearly a hundred cases of Typhus Fever I have only found it positive twice" (Ker, 1909). "The serum of a patient suffering from typhus does not clump typhoid bacilli" (Goodall and Washbourn, 1908). "The Gruber-Widal agglutination test will enable us in many cases to differentiate the two diseases" (Curschmann, 1902). "The agglutination test will establish the diagnosis of enteric fever" (Moore, 1906). "The Widal reaction and blood cultures are important aids" (Osler, 1909). Love (1905) mentions that in Typhus Fever a positive Widal reaction is sometimes obtained. Iversen (1905) obtained a negative Widal with 10 cases of Typhus Fever whose blood he tested. Galesesco and Slatinéano (1906) found that the serum of their patients never agglutinated Eberth's bacillus, *B. coli communis* or paratyphoid bacilli.

The results obtained by Horiuchi, Patterson and ourselves definitely prove that in Typhus Fever agglutinins for the typho-coli group of micro-organisms are present in the blood serum of the patients, but the knowledge which has recently been acquired with regard to the presence of heterologous agglutinins in cerebro-spinal fever prevents us from drawing the unwarranted conclusion that the presence of a bacillus in the intestine and urine and the discovery of agglutinins for it in the blood indicate that such an organism is the cause of the disease in question. Though future research may show that the causative organism of Typhus Fever is in no way related to diplococci or to any variety of intestinal organism still the presence of agglutinins for these organisms in the serum probably indicates that the latter are infecting the patient. We are now beginning to learn that the body in infection has not only to deal with the specific microbe and its toxins but also

with certain organisms contained in the alimentary canal which are normally saprophytic but which in the altered conditions of metabolism produced by disease become to some degree pathogenic.

VI.

The origin of Typhus Fever and the manner in which it is spread.

All observers agree that Typhus Fever is a disease which is always associated with filth, overcrowding and privation. Some observers, such as Murchison and Griessinger, maintained that these conditions were sufficient to cause the disease to arise spontaneously. When a case of the disease is once established the cases following can be shown in nearly all instances to have been exposed to infection direct or indirect of other cases. In one of the Belfast cases the virus appeared to have been carried in a shawl. In Belfast the infection could as a rule be traced to previous cases but "a number remained in which (to quote the words of Dr Bailie, M.O.H. Belfast, 1908) it was quite impossible to trace the infection, so obscure were they that one was almost compelled to give consideration to the old hypothesis of Murchison, *i.e.* spontaneous or *de novo* origin of Typhus Fever." Osler (1909) refers to outbreaks in which the source of infection could not be found.

The recently established facts regarding "Typhoid carriers" have served to clear up the origin of many outbreaks of enteric fever which in former times would have been taken as presenting clear evidence for the *de novo* theory. Kelsch (1893) believed that tramps might convey Typhus Fever although themselves healthy. Newsholme (1908) has shown from the Irish figures the close relationship that exists between Typhus Fever and poverty; vagrancy in his opinion was the great factor in the dissemination of the disease.

How poverty, overcrowding and bad sanitation cause Typhus Fever may be explained in three ways: (1) that these factors diminish the resisting power of the body to the action of a specific virus which is not generated by the conditions; (2) that these conditions may lead to the development of the virus either in the environment of the first person affected or in his alimentary canal. Such an unhygienic mode of life might lead to an alteration of the flora of the person's alimentary canal with the resulting possibility of certain germs acquiring the power of infecting the patient. Kelsch is a modern upholder of this hypothesis. In this connection w

may recall the fact that agglutinins for typhoid bacilli and colon bacilli are present in increased amounts in the serum of Typhus Fever cases; (3) that in such conditions the virus has the necessary means for its propagation, *e.g.* close atmosphere, presence of vermin, etc.

The majority of observers till recently believed that the virus clung to infected objects in the immediate vicinity of the patient and was inhaled entering the body through the respiratory tract.

Chantemesse (1893) and Curschmann (1902) favoured this view. Netter (1893) believed that the virus was swallowed and from the alimentary canal entered the blood: he also mentioned the possibility of the disease being conveyed by the bite of insects. The view that the contagium of Typhus Fever is conveyed from the patient to the healthy by fleas was strongly advocated on epidemiological grounds by Hay (1907).

Nicolle, Comte and Conseil (1909) in Tunis, after passage of the virus through a chimpanzee, were able to infect a monkey (*Macacus cynicus*) and from this to transmit the disease to two other monkeys by means of lice which had fed on the infected monkey and were then allowed to bite the healthy animals. Nicolle considered the possibility of bugs and fleas conveying the disease and came to the conclusion that the incidence of the disease could best be explained by inculcating the body louse. Anderson and Goldberger (1910) in Mexico also found evidence to incriminate body lice as the carriers of infection though their attempts to infect monkeys with Typhus Fever by means of them failed.

CONCLUSIONS.

(1) A relative increase in the large mononuclear leucocytes especially towards convalescence is very characteristic of the blood of Typhus Fever cases.

(2) During the febrile period the eosinophile cells are absent or very scanty in numbers. In two cases an eosinophilia was observed during convalescence.

(3) The blood of 33 different cases was examined by cultural methods. In 18 no growth of micro-organisms occurred whilst in 15 characteristic diplococci were cultivated. These diplococci were agglutinated by the blood serum and the agglutinins could be removed from the serum by saturating it with the cocci.

(4) From the faeces of one case a variant form of *B. coli communis* was cultivated on which the blood serum of 17 Typhus Fever cases was found to have 3 to 10 times the agglutinative effect of normal serum.

(5) From the urine of two cases a bacillus resembling *B. coli communis* but having no action on lactose was cultivated. This bacillus was agglutinated in dilutions of 1:50 and 1:100 by the serum of the cases but not by normal serum.

(6) The facts established by Anderson and Goldberger which showed that the virus of Typhus Fever was present in blood which afforded no growth on ordinary media suggest that the above micro-organisms are secondary invaders.

(7) Of 35 cases examined the blood serum of 19 gave a positive Widal reaction with the *B. typhosus*. Hence this reaction is of little or no value in differentiating Typhus from Typhoid Fever.

(8) The recent work of Anderson and Goldberger, Ricketts and Wilder, Nicolle, Comte and Conseil goes far to prove that the virus is present in the blood and that insects, probably *Pediculi vestimenti*, are the agents by which infection is carried.

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ON THE DIFFERENTIATION OF PROTEINS OF CLOSELY RELATED SPECIES BY THE PRECIPITIN REACTION.

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THE ultimate problem underlying many applications of the precipitin test, whether it be the determination of biological relationships, the identification of blood stains and other animal traces, or the detection of adulteration in food, is the recognition of the homologous protein (antigen) and its separation from closely allied heterologous proteins. Recognising that the antiserum is the main source of the precipitate in a precipitin reaction and having regard to the exact quantitative relations of antiserum, antigen and precipitate we have been able to arrange methods for the differentiation of proteins of closely related species and, we believe, to render more accurate the diagnosis of the source of individual proteins. To take a crucial instance, by means of an antiserum prepared with hen egg-white we have been able clearly to distinguish solutions of hen egg-white from all other avian egg-whites tested, including those of the duck, quail, partridge, pheasant and ostrich. So far as we know, Nuttall and Graham Smith alone have previously been successful in differentiating homologous and heterologous avian egg-albumens, and their methods appear to be more cumbersome than ours. Incidentally we have found that our results are not only consistent *inter se* but consistent also with the interpretation of the precipitin reaction which our previous observations had led us to adopt.

Historical. In 1900 Myers⁽¹⁾ and, a few months later, Uhlenhuth⁽²⁾ demonstrated that antisera, prepared by injecting rabbits with hen egg-albumen, yielded precipitates when added to avian egg-albumens,

the precipitation being more marked with homologous than with heterologous egg-albumens. In 1901 Uhlenhuth⁽³⁾ found that a precipitin antiserum prepared with hen egg-white might cause precipitation in solutions of avian blood sera as well as in solutions of avian eggs. Further, by injecting a rabbit with goose egg-white, he obtained an antiserum which gave abundant and rapid precipitation with goose and duck eggs and well marked clouding with hen, guinea-fowl and pigeon eggs. He was led to conclude that it was not possible to distinguish the various kinds of eggs by the precipitin test, as he had done with different blood sera. In 1902 Gengou⁽⁴⁾ stated that he could not observe any difference in the action of hen egg-white antiserum upon solutions of the egg-whites of the hen, duck, pigeon and turkey.

Nuttall, in a series of publications^(5,6,7), confirmed and extended the original observations of Myers and Uhlenhuth, obtaining positive reactions with a hen-egg antiserum and a variety of avian and reptilian bloods, suggesting the "reptilian-avian" character of the reaction. He also introduced a "quantitative method for the measurement of the degree of the reaction" by estimating the bulk of the precipitate from measured quantities of the interacting dilutions.

In 1904 Nuttall⁽⁸⁾ recorded two hen-egg antisera and one emu-egg antiserum, each of which gave the largest reaction only with the corresponding homologous protein and lesser reactions with other avian eggs and with some avian and reptilian blood sera. At Nuttall's suggestion Graham-Smith⁽⁹⁾ extended his work with the result that their qualitative methods proved to be inadequate to distinguish the homologous from heterologous egg-whites, but Nuttall's quantitative method was successful in every case in which it was tested.

In an earlier paper⁽¹⁰⁾ we summarised other methods that had been proposed for the differentiation of closely allied proteins by Nuttall, by Linossier and Lemoine, by Ewing, by Weichardt and by Uhlenhuth. Ewing⁽¹¹⁾, following a suggestion made by Uhlenhuth and others, tried the effect of progressively diluting the antiserum while maintaining the blood dilutions constant. He found that when added to various bloods in solutions of equal strength an antihuman serum in its highest dilution acted only upon human blood dilutions, and his other results were concordant.

In the same paper⁽¹⁰⁾ we published a preliminary account of a method which we had independently devised, and which, though superficially similar, is fundamentally different from that of Ewing. To a series of

fixed quantities of each protein to be tested there were added progressively diminishing amounts of the antiserum. The quantities of protein and of antiserum were regulated by the consideration that the quantity of protein, when homologous, should be sufficient and not much more than sufficient, to give a maximum precipitate with the greatest amount of antiserum employed.

Our method was based on the experimental finding that the precipitable substance is contained in the antiserum^(10, 12, 13) and that there is a quantitative relation between the amount of precipitate and the amount of antiserum, provided the homologous protein is sufficient. Since then we have obtained experimental evidence^(14, 15) that the antigen being in sufficient amount the weight of precipitate is proportional to the weight of antiserum engaged in the interaction. This last observation places the method on a scientific basis and offers some guarantee of its accuracy.

Experimental. We start from the experimental result that in certain conditions a given quantity of antiserum yields a definite weight of precipitate, provided that a sufficient amount of homologous protein be present. If the protein of the homologous species be replaced by the protein of any heterologous species, however closely related (as tested by the biological method), the weight of precipitate from that quantity of antiserum is diminished. It is not, however, generally practicable to weigh the precipitate from a given quantity of antiserum interacting with a quantity of unknown protein as a means of differentiation of proteins. But the same principle, adapted to other circumstances, may be employed to distinguish between closely related proteins. As an example we shall quote experiments which record the interactions between antisera for hen egg-white and the egg-whites of the hen, duck, quail, partridge, pheasant and ostrich, and by which the heterologous egg-whites of the different eggs were clearly distinguished from hen egg-white.

The antiserum, derived from a rabbit which had received six injections of hen egg-white (altogether equivalent to 6.27 gm. dried egg-white), was dried in vacuo over calcium chloride at 37° C. At the time the experiments were performed the antiserum had been dried for over two months. In the first experiment diminishing amounts of the antiserum were allowed to interact with constant quantities of the homologous and heterologous proteins. The antiserum solution was prepared by dissolving 0.13 gm. dried antiserum in 5.2 c.c. saline solution, so that 0.4 c.c. of the solution contained 0.01 gm. dried

antiserum, 0.2 c.c. solution contained 0.005 gm. antiserum, and so on. Solutions of the various egg-whites were obtained by diluting 1 c.c. of egg-white from each of six kinds of eggs (hen, duck, quail, partridge, pheasant and ostrich) with 99 c.c. saline solution; and 0.1 c.c. of the solution of each kind of egg-white was placed in each of six tubes, so that six series of tubes were arranged, each series consisting of six tubes. The original antiserum solution was measured out in quantities equal to six times that required for each tube, and saline solution added in such quantity that the amount of diluted antiserum for six tubes measured 3 c.c. in all. Of this secondary dilution of antiserum 0.5 c.c. was transferred to each tube. In this way it was possible to measure the small amounts of antiserum with some approach to accuracy. The quantities of the interacting bodies in each series of tubes are given in Table I.

TABLE I.

No. of tube in each series	Weight of dried antiserum	Amount of the original solution of antiserum	Amount of saline solution added to original solution of antiserum	Amount of diluted egg-white (hen, duck, quail, partridge, pheasant and ostrich)
1	0.01 gm.	0.4 c.c.	0.1 c.c.	0.1 c.c.
2	0.005	0.2	0.3	0.1
3	0.002	0.08	0.42	0.1
4	0.001	0.04	0.46	0.1
5	0.0005	0.02	0.48	0.1
6	None	None	0.5	0.1

In conducting the experiments, precautions were taken against bacterial contamination. The reactions were allowed to take place at room temperature (about 18° C.) and the precipitates were read after 48 hours, as given in Table II.

TABLE II.

No. of tube in each series	Weight of dried antiserum	Precipitate with hen egg-white	Precipitate with duck egg-white	Precipitate with quail egg-white	Precipitate with partridge egg-white	Precipitate with pheasant egg-white	Precipitate with ostrich egg-white
1	0.01 gm.	2.5 mm.	1.0 mm.	0.8 mm.	0.8 mm.	1.0 mm.	0.5 mm.
2	0.005	1.0	0.3	0.3	0.5	0.5	0.3
3	0.002	0.3	trace	trace	trace	trace	trace
4	0.001	trace	trace	none	none	trace	none
5	0.0005	trace	none	none	none	none	none
6	none	none	none	none	none	none	—

The results show that the precipitate with hen egg-white was much greater than the precipitate with any heterologous protein, and that the differentiation is easily made by testing in this way with diminishing

quantities of antiserum. Although 43 tubes were employed (including controls), the amount of dried antiserum required was only 0.13 gm., equivalent to 1.3 c.c. fresh antiserum. The method is therefore economical of material.

An unknown protein solution could be made comparable with the 1 % protein solutions above employed by so adjusting the dilution that 0.1 c.c. should yield with trichloroacetic acid a precipitate measuring between 1 mm. and 2 mm. in narrow tubes, as described by us ⁽¹²⁾. Then 0.1 c.c. of the unknown protein solution would contain approximately 0.0001 gm. of dried protein; and the test could be carried out by comparing this solution with similar dilutions of the homologous protein, and of a closely allied heterologous protein.

Further experiment showed that, when the quantity of heterologous protein interacting with 0.01 gm. dried antiserum is increased to produce the maximum precipitate obtainable from that amount of antiserum, the amount of precipitate is less than the full precipitate yielded by the same amount of antiserum interacting with a sufficiency of the homologous protein. An illustrative experiment is given in Table III which records the result of an experiment similar to that quoted in Tables I and II but carried out with another hen-egg antiserum. At the end of every 48 hours the superfluids were removed to clean tubes and treated with a fresh amount (0.1 c.c.) of the corresponding solution of egg-white.

TABLE III.

No. of tube	Weight of dried hen-egg antiserum	Amount of diluted egg-white	Precipitate in 48 hours	Addiment to superfluid of diluted egg-white	Precipitate from superfluid in 48 hours
1	0.01 gm.	0.1 c.c. (hen)	2.5 mm.	0.1 c.c. (hen)	0.3 mm.
2	0.01	0.1 c.c. (duck)	0.5	0.1 c.c. (duck)	none
3	0.01	0.1 c.c. (ostrich)	0.5	0.1 c.c. (ostrich)	0.5 mm.

Further addiments of 0.1 c.c. of the respective egg-white solutions to the superfluids produced no further precipitation. The readings show that the combined precipitates obtained with any heterologous protein did not equal the combined precipitates given by the homologous protein.

Another method of differentiating closely allied proteins has been described by us ⁽¹⁶⁾. This method depends on the inhibition of the formation of precipitate by heated antisera, and particularly on the phenomena of "crossed inhibition." It is not, however, so simple as that described above, as it involves a knowledge of the inhibitory powers

of the antisera employed, and requires a detailed examination of each antiserum before use. The results obtained in our work on "crossed inhibition" led us to suggest that the precipitate given by hen-egg antiserum and ostrich or any egg-albumen other than hen egg-albumen might be regarded as similar to that produced by ostrich-egg antiserum and any egg-albumen other than ostrich egg-albumen. It could be assumed that this precipitate resulted from the general avian character or component of the proteins used in the immunisation, while the increased precipitate produced by hen egg-albumen and hen-egg antiserum, or by ostrich egg-albumen and ostrich-egg antiserum, could be assumed to be due to the specific hen or ostrich character or component of the material used for injection.

In this connection some observations made on the eggs used for the experiment quoted in Tables I and II may be noted. After 48 hours the reactions recorded in Table II were completed, and the precipitates were read. The superfluids of tubes No. 1 in each series were removed to clean tubes, and to these superfluids certain addiments of the solutions of egg-white were made. The solutions were those used in the original experiment. The observations are detailed in Table IV, where the first four columns are merely a rearrangement of certain data from Tables I and II.

TABLE IV.

No. of tube in Tables I and II	Weight of dried hen-egg antiserum	Amount and nature of the original 1% solu- tion of egg-white	Precipitate at 48 hours	Amount and nature of the 1% solution of egg-white added to clear superfluid	Precipitate from superfluid at 48 hours
1 (hen series)	0.01 gm.	0.1 c.c. (hen)	2.5 mm.	0.1 c.c. (ostrich)	none
1 (duck series)	0.01	0.1 c.c. (duck)	1.0	0.1 c.c. (ostrich)	trace
1 (quail series)	0.01	0.1 c.c. (quail)	0.8	0.1 c.c. (partridge)	none
1 (partridge series)	0.01	0.1 c.c. (partridge)	0.8	0.1 c.c. (hen)	1.5 mm.
1 (pheasant series)	0.01	0.1 c.c. (pheasant)	1.0	0.1 c.c. (partridge)	0.5 mm.
1 (ostrich series)	0.01	0.1 c.c. (ostrich)	0.5	0.1 c.c. (duck)	0.5 mm.

In the interpretation of these results the observations noted in Table III must also be considered. There it is seen that one addiment of the heterologous protein solution is sometimes sufficient to neutralise the whole of the general avian precipitin present (cf. tube No. 2); whereas in other cases a single addiment of the heterologous protein solution does not suffice (cf. tube No. 3). On our interpretation of the precipitin reaction this is equivalent to saying that in some cases a single addiment of heterologous protein suffices to throw out of solution the whole of the general avian precipitable content of the antiserum;

whereas in other cases the whole of the general avian precipitable substance is not so discharged.

Among the results of Table IV similar phenomena appear. In the superfluids of the duck and quail series the addition of a different heterologous protein failed to reveal a precipitate, probably because the general avian precipitable content (precipitin) had been completely discharged in the previous interaction; whereas in the superfluids of the pheasant and ostrich series the additional different heterologous protein revealed the presence of some general avian precipitable substance (precipitin) remaining undischarged after the primary interaction. In the superfluid of the hen series the addition of ostrich egg-white failed to yield a precipitate, probably because the primary interaction with the homologous protein had completely eliminated the general avian precipitable substance from the antiserum together with most of the specific hen "precipitin." In the superfluid of the partridge series the addiment of hen egg-white precipitated the specific anti-substance (precipitin) for hen egg-white, giving therefore a large precipitate, the primary interaction with a heterologous protein having affected only the general avian antisubstance.

These results have a further interest in the light of similar "saturation phenomena" that may be exhibited by haemolytic antisera.

In order not to load our paper with experimental detail we have quoted only a few of our observations in illustration of our points. But they are supported by many similar experiments which we have carried out at different times and always with concordant results.

CONCLUSIONS.

(1) It is possible clearly to distinguish heterologous proteins of closely related species from the homologous protein by precipitin interactions arranged with regard to the fact that in the conditions of the experiment the weight of precipitate is proportional to the weight of antiserum employed.

(2) By "saturation experiments" it is possible to indicate in an avian egg-white antiserum the presence of a general avian antisubstance (precipitin) together with the specific antisubstance.

(3) The consistency of these results with our interpretation of the precipitin reaction lends further support to the working hypothesis which we have advanced in previous papers.

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ON THE HAEMOLYTIC IMMUNE ISOLYSINS OF THE OX AND THEIR RELATION TO THE QUESTION OF INDIVIDUALITY AND BLOOD-RELATIONSHIP.

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THE term "Isolysin" was first employed by Ehrlich and Morgenroth in the third of their now classical series of papers entitled "Studies on Haemolysis⁽¹⁾." It had been shown by Bordet that if the red blood corpuscles of an animal A are injected into another animal B, of a different species, the serum of B develops a haemolysin for the corpuscles of A. This haemolysin being produced in a different species of animal from that yielding the corpuscles, is termed, according to Ehrlich's nomenclature, a heterolysin.

Ehrlich and Morgenroth now set themselves to investigate the results of injecting animals with the red blood corpuscles of other animals of the same species and found that here again a haemolysin was produced which they termed an *isolysin*, as it was formed in animals of the same species as those providing the corpuscles.

Ehrlich and Morgenroth were led to these researches by the following considerations, which are perhaps best given in their own words :

"In pathology, the changes foremost to be considered are those resulting from the absorption, by an organism, of its own cell material. Such occasions are presented by many different diseases. Keeping to the blood, for example, if an individual suffers a considerable subcutaneous haemorrhage or one into a body-cavity, or if part of his blood-corpuscles are destroyed and dissolved by certain blood-poisons, the

essential conditions, just as in experiment, are given for the reactive formation of substances possessing specific injurious affinities for these blood-cells.....

"It is therefore of the highest pathological importance to determine whether the absorption of its own body material can excite reactive changes in the organism, and what the nature of these changes is. The simplest conditions and those most accessible to experimental study are those which arise on the absorption of blood-cells. But here we face a curious dilemma. If an animal organism when injected with blood-cells of foreign species always produces a specific haemolysin for each of these species it must surely be following a natural law; and it is improbable that this law which applies in any particular number of cases should be suspended in the case of blood-cells of the same individual. On the other hand it is not to be denied that the formation of such haemolytic substances would appear dysteological in the highest degree. For example, if, in an individual who has had an extensive haemorrhage into a body-cavity, the absorption of this blood caused the formation of a blood-poison which destroyed the rest of the blood-cells, this would be a phenomenon whose actual occurrence lacks any clinical evidence whatever and one which no one is willing to accept.

"It cannot be doubted that the organism seeks a way out of this difficulty by means of certain regulating contrivances, whose determination will be of the highest interest."

In order to investigate this question Ehrlich and Morgenroth immunised a series of goats with goats' blood. This was done by giving one intraperitoneal injection of a somewhat large volume of blood which had been previously laked by the addition of water. Laked blood was employed as it was thought that the uninjured blood corpuscles of the same species as the animal injected would be destroyed very slowly in the peritoneal cavity of this animal, and that consequently the absorption would be so gradual as to prevent the occurrence of what may be termed an "ictus immunatorius."

It was found in this way that the injection of goats' blood into other goats resulted as a rule in the formation of isolysins, but that in no instance was an autolysin formed, *i.e.* the serum of the injected animal never acquired the property of dissolving its own corpuscles. Altogether thirteen isolytic sera were prepared and their characters studied by means of anti-isolytic serum with the somewhat astonishing result that it was found that they all differed from one another, *i.e.* that they represented different isolysins.

The great interest presented by these isolysins rendered a more extensive examination of their nature and properties most desirable; but the immunisation of a large number of animals in this way is most tedious. Fortunately, however, the writers happened to have at their disposal, in the Institute for the preparation of cattle plague serum at Cairo, about 100 animals which in the course of their immunisation with cattle plague had received large quantities of cattle blood under almost ideal conditions for the formation of isolysins.

These cattle, which were used for the preparation of cattle plague serum, were immunised by Kolle and Turner's method, consisting in a preliminary simultaneous inoculation of cattle plague immune serum and a small quantity of virulent cattle plague blood, followed after a short interval by an intramuscular injection of four litres of virulent blood. These massive injections of four litres were repeated regularly every two months as long as the animal was used for the production of serum.

The cattle were bled for serum 14 days after the large injection of blood and again three times at intervals of a fortnight before the next injection. The animals used for the production of serum were Egyptian cattle and the blood used for their immunisation was that of freshly imported Cyprus cattle. On testing the fresh serum of the cattle which had been immunised in this way on the red blood corpuscles of Cyprus and Egyptian cattle it was found that it showed practically no haemolytic action; but that on the addition of a little fresh guinea-pig serum, it became powerfully haemolytic, the lack of haemolytic action of the fresh serum being due only to the want of a suitable complement. This point appears to have been overlooked by Frei⁽²⁾, who in referring to cattle plague serum says:

"Only the specific rinderpest anti-bodies were observed, even after the hyperimmunisation of oxen with great quantities of virulent rinderpest blood in order to obtain a strong immune serum, recognised *in vivo* by the successful treatment of animals; in no instance were clinical or anatomical phenomena due to haemolysis or precipitation recorded. We are therefore confronted by the remarkable fact that cattle do not produce isolysins in their blood, *i.e.* substances with the property of dissolving cattle blood *in vivo* or *vitro*, even after the injection of enormous quantities of blood."

In order to get an approximate idea of the strength of the sera of the various animals, a rough test was made of the sera of all the serum producing animals in the Institute, the haemolytic dose being taken as

the amount of the serum required to haemolyse in 1 hour at 37° C. 1 c.c. of a 5 % suspension of the corpuscles of one normal Cyprus bull in the presence of 1/10 c.c. of fresh guinea-pig serum.

The results were as follows :

4 animals showed no haemolysis at 1/10 c.c.			
26	„	„	complete haemolysis at 1/10 c.c.
76	„	„	complete haemolysis at 1/100 c.c.
<hr/>			
106			

A certain number of sera were also tested at 1/1000 c.c., but in no case was complete haemolysis obtained at this dilution.

The minimum haemolytic dose may therefore in the majority of cases be said to lie between 1/100 and 1/1000 c.c.

If the serum of one immune animal is tested on the red blood corpuscles of a series of different individual cattle marked differences are noted in the susceptibility of the corpuscles of the different individuals, some being highly susceptible, others less so, and others again very resistant.

If now the serum of a second immune animal is tested on the same series of corpuscles similar differences are noted but these are generally not parallel to those obtained with the first serum. Corpuscles which are highly susceptible to one serum may be resistant to the other and vice versa.

This is shown in the following table :

TABLE I.

Showing action of sera of immune cattle on corpuscles of the same individuals.

		Immune serum	0·03 c.c.			
		Fresh guinea-pig serum	0·10 c.c.			
		5 % suspension of ox corpuscles	1·0 c.c.					
		Red blood corpuscles of ox						
		No. 80	No. 86	No. 90	No. 92	No. 99	No. 102	
Immune serum of ox	{	No. 80	0	+++	+	tr.	tr.	+
		No. 86	++	0	+++	+	+++	+
		No. 90	+++	0	0	+	+++	+
		No. 92	+++	+++	+++	0	+++	+++
		No. 99	+++	+++	+++	+	0	+++
		No. 102	0	0	0	0	0	0
		tr.	=	trace of haemolysis.				
		+	=	definite „				
		++	=	marked „				
		+++	=	complete „				

These results are quite in accordance with what was found by Ehrlich and Morgenroth in their isolytic goat sera.

It is interesting to note that the corpuscles of the immune animals did not, on the whole, appear to show any greater resistance than those of fresh animals, and also that the question of race seemed to have no marked effect. The animals were immunised entirely with the blood of Cyprus cattle, but in spite of this their sera appeared to act equally strongly on the corpuscles of Cyprus, Egyptian and Soudan cattle.

The haemolytic action of the serum of the immune cattle was not limited to the corpuscles of the ox but also acted very powerfully on those of the sheep and goat. The corpuscles of the Egyptian buffalo, camel, rabbit, guinea-pig and man were not affected, at any rate in the presence of guinea-pig complement.

The haemolysin in the serum of the immune cattle is an isolysin, but appears to be practically never an autolysin, *i.e.* it has no action on its own corpuscles. Of all the sera examined by us only in one case was haemolysis observed in the tube containing the immune serum with its own corpuscles, and in this case the amount of haemolysis was slight and may have been due to some accidental circumstance. As a general rule the tube containing the immune serum with its own corpuscles was sharply picked out by the complete absence of even the faintest trace of haemolysis.

This result is in complete accord with what was found by Ehrlich and Morgenroth in their isolytic goat sera.

In the course of our investigations we were much puzzled by the fact that the isolysin present in the serum of the immune cattle does not act with the complement free in the blood of these cattle but appears to require a foreign complement.

We naturally assume that the formation of the isolysin is protective and has for its object the solution and removal of the strange corpuscles; hence in the case of the goat the injection of the animal with the corpuscles of another individual gives rise to an isolysin which, acting in conjunction with the complement normally present in the serum of the goat, causes the solution of the corpuscles which are then easily dealt with.

In the case of the ox, however, we have the formation of an isolysin which has practically no action on ox corpuscles in the presence of fresh ox serum.

It may be added that, by means of a different haemolytic system, it was easy to demonstrate the presence of complement in this serum.

As it was difficult to imagine that the organism would, so to speak, go to the trouble of elaborating a complex isolyisin which would not act with the complement available, we were led to enquire whether a suitable complement did not exist elsewhere in the body and with this idea the following experiment was made:

A normal Cyprus bull was injected intravenously with one litre of the mixed serum of ten immune cattle. This serum, although only 24 hours old, showed no action on ox corpuscles *in vitro* if no foreign complement were added, but in the presence of fresh guinea-pig serum was very powerfully haemolytic (0.01 c.c. being sufficient to haemolyse 1 c.c. of 5% suspension of ox corpuscles). A few hours after the injection the urine was very darkly haemoglobin stained, showing that a suitable complement had been forthcoming.

On testing the serum of the animal a few days later it was found that although it had now no haemolytic action on its own corpuscles it was distinctly haemolytic for the corpuscles of many other individuals, *i.e.* it was isolytic but not autolytic. It was thus possible, by passive immunisation with the serum, to produce a condition apparently similar to that of the animals actively immunised with corpuscles.

A series of experiments¹ were now made by replacing this "exhaustion" of the serum *in vivo* by exhaustion *in vitro*, the technique being as follows:

The immune serum was mixed with an equal volume of the washed corpuscles with which it was desired to exhaust the serum; the mixture kept at 37° C. for an hour, centrifuged, and the serum again treated in the same way with more washed corpuscles and the process repeated a third time. It was then found that the serum had lost all traces of haemolytic power for the corpuscles in question.

By means of this method the sera of different immune cattle were now exhausted with the corpuscles of various individuals of the same species and the haemolytic power of these sera, after such treatment, was studied on the corpuscles of different individuals.

It was found that if an immune serum is exhausted with corpuscles of an individual (A) it remains haemolytic for the corpuscles of many other individuals, but loses its haemolytic power for the corpuscles of some other individuals as well as for those of (A).

If now a second immune serum is exhausted with the same corpuscles (A) its action on the various corpuscles is not exactly

¹ A preliminary note of these experiments was published in the *Proceedings of the Royal Society*, June, 1910.

parallel to that of the first serum and often shows very marked differences.

This result is to be expected, as it was shown by Ehrlich and Morgenroth that two goats each injected with similar doses of the same goat's blood at the same times gave quite different isolysins. In fact the isolysins formed depend upon two distinct factors:

- (a) the individuality of the injected corpuscles,
- (b) the individuality of the animal into which they are injected.

When we consider the enormous number of variations possible in each of these factors we see the almost unlimited possibilities in the resulting sera.

In view of the above it should be possible by taking a mixture of a sufficiently large number of immune sera and exhausting this with the corpuscles of one individual, to obtain a serum which is specific for the corpuscles of this one individual, *i.e.* which has no haemolytic action on these corpuscles, but haemolyses those of all other individuals of the same species. To test this, a mixture was made of the sera of between 60 and 70 immune Egyptian cattle. This mixture was then exhausted with the corpuscles of a normal Cyprus bull and then tested on the washed corpuscles of 20 immune Egyptian cattle, two normal Cyprus cattle and the above mentioned Cyprus bull with whose corpuscles the mixture had been exhausted. For the test equal parts were taken of

- (a) the exhausted serum,
- (b) a 5 % suspension of the washed red blood corpuscles,
- (c) a one-tenth dilution of fresh guinea-pig serum in normal saline.

The tubes were kept at 37° C. for one hour and then left over-night in the ice-safe; after which the results were read off.

It was found then that complete haemolysis had occurred in all the tubes, with the exception of the control tube containing corpuscles of the Cyprus bull with which the serum was exhausted. The exhausted serum was thus able to pick out, quite sharply, the corpuscles of one individual from those of 22 others. Following up these results, a second and more extensive test was made. In this case the same immune serum was used; it was, however, exhausted with the corpuscles of another normal bull.

This exhausted serum was then tested on the corpuscles of 110 different cattle (3 Soudan, 34 Cyprus, 73 Egyptian).

In this test again the tubes showed complete haemolysis with the exception of the one containing the corpuscles for which the serum had been "exhausted." This control tube showed no trace of haemolysis.

A number of other tests has been made by exhausting the serum with the corpuscles of various individuals, and the general rule has so far always held except in the case of close blood-relations, where certain exceptions occur which will be dealt with later.

These results show that the red blood cells of any individual (excluding for the moment the question of close blood-relations) possess characters which differentiate them quite distinctly from the red blood cells of any other individual even of the same species¹.

A consideration of this very striking fact at once leads to speculation as to whether this is not merely one example of a general law and if all the cells of an individual are not, so to speak, stamped with his individuality. We know that this holds for the spermatozoon and the ovum and it can be experimentally demonstrated for the red blood corpuscles, but whether it will prove to be the case for other cells remains to be determined. Unfortunately the other cells present much greater experimental difficulties than the red blood cell whose delicate stroma renders it an ideal object for such observations.

Another point which the above results emphasize is the enormous complexity of the structure of the red blood cells which renders it difficult to imagine that these cells have no other function than the comparatively simple one of acting as oxygen carriers. This question has been pointed out by Ehrlich⁽³⁾ in his exceedingly suggestive article on "The receptor apparatus of the red blood corpuscles," where he suggests that these cells may be regarded as storage reservoirs in the sense that they temporarily take up the most varied substances derived from the food or from the internal metabolism.

Having found that it was possible to distinguish the red blood corpuscles of non-related individuals of the same species, it was interesting to compare the corpuscles of closely related individuals. The first test was made on the corpuscles of a cow and her calf. The mixed sera of a considerable number of immune cattle were exhausted with the corpuscles of the mother and calf separately and it was found that while

¹ v. Dungern and Hirschfeld (*Zeitschr. f. Immunitätsforschung*, iv., p. 531), using a similar method, have recently investigated the red blood corpuscles of dogs by means of iso-agglutinins. They come to the conclusion that dogs may be divided into several groups according to the agglutination of their corpuscles and conclude that the red blood corpuscles of all the animals belonging to one group have an identical chemical structure.

exhaustion of the serum with the corpuscles of the calf removed the haemolysin for the calf only, exhaustion of the serum with the corpuscles of the cow removed the haemolysin not only for the cow but also for the calf. The continuance of these investigations was much hampered by the difficulty of obtaining, in Egypt, samples of blood from the various members of complete families of cattle or goats; but by the kindness of Mr Littlewood, C.V.I., we had the opportunity of examining an interesting group of sheep. This consisted of three ewes each with one lamb; all the lambs were by the same father, which was fortunately available.

The first test was made on the corpuscles of the father (tup), ewe A, her lamb (lamb *a*) and another sheep not related to the family in question.

A mixture of the sera of about 80 immune animals was twice exhausted with an equal volume of the various corpuscles, and the serum then tested separately on the various corpuscles in the presence of guinea-pig complement as follows:

TABLE II.

Showing action of polyvalent serum, after exhaustion with sheep's corpuscles, on the corpuscles of closely related sheep.

Exhausted immune serum ... 0.3 c.c.
 $\frac{1}{10}$ dilution of fresh g.-pig serum ... 0.3 c.c.
5 % suspension of sheep's corpuscles 0.3 c.c.

		Red blood corpuscles of			
		Tup (father)	Ewe A (mother)	Lamb <i>a</i> (child)	Non-related pregnant sheep
Immune serum exhausted with corpuscles of	Tup (father)	0	+++	+++	+++
	Ewe A (mother)	+++	0	0	0
	Lamb <i>a</i> (child)	++	0	0	0
	Non-related pregnant sheep	+++	+++	++	0

It will be noticed that there is a marked parallelism between the lamb and ewe and none between the lamb and father.

Next day the test was continued on the rest of the group as follows (Table III).

On examining the results of the whole test it is seen that while the corpuscles of lamb (*a*) resemble exactly those of the mother, the corpuscles of lambs (*b*) and (*c*) show no resemblance to their mother's but almost exactly resemble those of their father.

TABLE III. Continuation of Table II.

		Red blood corpuscles of								Non-related sheep		
		Tup	Ewe A	Lamb a	Ewe B	Lamb b	Ewe C	Lamb c	(1)Tup	(2)Ewe	(3)Ewe	
Immune serum exhausted with corpuscles of	Tup	0	+++	+++	+	0	+++	0	+	0	++	
	Ewe B	+	+	0	0	0	++	+	0	0	0	
	Lamb b	+	+++	+++	++	0	+++	+	+++	0	0	
	Ewe C	++	0	0	0	0	0	+	0	0	0	
	Lamb c	0	+++	+++	++	0	+++	0	++	+	+	
	Non-related sheep :											
	(1) Tup	++	+	+	+	0	+++	+	0	0	0	
(2) Ewe	+	++	+	+	+	++	0	+	0	0		

This result is analogous to those obtained by v. Dungern and Hirschfeld⁽⁴⁾ who by means of their isoagglutinins found that the offspring of two dogs whose blood belonged to different biochemical groups showed different relations towards their parents. The blood of one pup belonged to the same group as the mother, the blood of another showed the structures of both parents, while two others showed no resemblance to either.

The problem is exceedingly complicated and much work will have to be done before it is safe to generalise, but we believe that the results obtained so far are sufficient to justify the hope that the method may be useful in attacking the question of heredity.

In conclusion we should like to acknowledge our indebtedness to Mr Allen, Veterinary Surgeon to the Serum Institute, who has rendered us invaluable assistance.

CONCLUSIONS.

1. The immunisation of the ox with the red blood corpuscles of other oxen gives rise to the formation of a haemolytic amboceptor in the blood of the immunised animals.
2. The amboceptor so formed is an *isolysin* but not an *autolysin*.
3. The amboceptor is fixed by susceptible corpuscles; it is not activated by the complement present in the fresh serum of the ox, but requires the addition of a foreign complement.
4. The race of the animal furnishing the corpuscles appears to have very little influence on the resulting haemolysis.

5. The serum of an animal so immunised acts very differently on the red blood corpuscles of different individual oxen.

6. The sera of different individuals similarly immunised differ from one another in their action on the corpuscles of different individuals.

7. If the serum of a single immunised animal be "exhausted" with excess of the corpuscles of one other individual, the serum loses its power of haemolysing the corpuscles of this individual, while retaining the power of haemolysing the corpuscles of many, but not all, other individuals.

8. If, however, a polyvalent serum be made by mixing the sera of a large number of immunised animals, and this serum is exhausted with the corpuscles of any one individual, the serum entirely loses its power of haemolysing the corpuscles of this individual, but remains strongly haemolytic for all other individuals not closely related to the individual whose corpuscles were employed for the exhaustion of the serum.

(N.B. It is possible that exceptions may be found, but these have not yet been met with except in the cases of close blood relations.)

9. The red blood corpuscles of any individual are thus characterised by a definite individuality of their own and can be distinguished from those of any other individual of the same species.

10. When studied by the above method the red blood corpuscles of closely related animals show interesting relations with one another, which would suggest the application of the method to the study of the question of heredity.

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REPORT ON EXPERIMENTS UNDERTAKEN TO DISCOVER
WHETHER THE COMMON DOMESTICATED ANIMALS
OF TERCEIRA ISLAND ARE AFFECTED BY PLAGUE.

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WE have recently had the opportunity of studying a plague epidemic that broke out on Terceira Island, Azores, in 1908. Terceira Island contains a population of 45,000 souls, the epidemic spread over 27 parishes. About 260 persons were attacked, with a mortality of about 48 %.

In the course of our study we have seen the conclusions of the *Reports on Plague Investigations in India*, which have appeared in Vols. VI., VII. and VIII. of this *Journal*, 1906, 1907 and 1908, fully justified, nevertheless the "Conselho Superior de Hygiene de Reino" resolved to prohibit the entry into the Portuguese continent of birds and pigs from Terceira Island, and exacted that the black cattle should be disinfected in order to be accepted.

This action of the Council was presumably based upon the opinion arrived at by Simpson (1905) from his experiments in Hong-Kong and given in his *Treatise of Plague* (1905). As, however, Simpson's experiments and conclusions are criticised by Pearse, Acting Medical Officer of Health for Hong-Kong (1904), and the work of Walton (1900) and Bannerman and Kapadia (1908), made in India, of London

(1897), made in Russia, of Watkins-Pitchford (1904), made in Natal is in complete disagreement with the results of Simpson, we have also made experiments in this matter.

The mammals of the island that we could get for our experiments were 2 calves, 8 pigs, 2 cats and 1 kid; the birds were 8 pigeons, 1 turkey, 3 ducks and 85 hens.

Very briefly we are going to describe our experiments and their results.

Calves.

Calf No. 1. Female, about 75 kilograms in weight. It received subcutaneously an emulsion in physiological salt solution of 14 tubes of agar culture of the plague bacillus, pure and virulent and of very recent human origin. The virulence was confirmed in guinea-pigs. The calf did not show the slightest sign of sickness, eating well and remaining lively. There was no local reaction. The inoculation took place on 1 January, 1909 and the calf was abandoned on 24 April, 1909.

Calf No. 2. Female, 60 kilograms in weight. On 12 January, 1909, maize meal was mixed with pieces of the organs from a case of septicaemic plague, and also those of a cat which died of bubonic plague. The animal would only lick this mixture a few times, therefore on the next day we administered by the mouth agar cultures (pure) of the plague bacillus obtained from five Petri dishes (11 cm. in diameter). The cultures were mixed with dry maize leaves, the customary fodder of the cattle in this island. No morbid alteration was remarked in the animal, which was abandoned on 24 April, 1909.

Pigs.

Pig No. 1. Black, male, 10 kilograms in weight. It was inoculated on 31 December, 1908, in the following manner:

We made a mixture of meal and maize corn with cold water, to which we added a decapitated rat, *Mus norvegicus*, cut into small portions. The rat had died of septicaemic plague, as was shown by the anatomo-pathological aspect and by the enormous quantity of bacilli present in the blood, spleen, liver, etc. On the following day the pig ate, mixed with pieces of raw potato, the liver, spleen, lungs, heart, kidneys and glands of another rat (*M. norvegicus*) which died of septicaemic plague, with an enormous quantity of bacilli in all the organs. On the next day, at two o'clock in the afternoon, the pig ate,

with mashed potatoes, the organs of a guinea-pig (spleen, liver, lungs, heart, kidneys, supra-renal capsules, serous haemorrhagic peritoneal fluid) that died two days after the peritoneal inoculation of a pure culture of plague obtained from the spleen of a rat (*M. norvegicus*) spontaneously infected. On the same day at eight o'clock the pig swallowed the viscera of a cat attacked by primary pneumonic plague and spontaneously infected. The pig showed no signs of illness, always ate well, and perceptibly fattened until abandoned on 15 February, 1909.

Pig No. 2. Female, black with white marks, Yorkshire breed, crossed with pigs of the island, weighing 15 kilograms. On 1 January, 1909, it was inoculated subcutaneously with an emulsion in physiological salt solution of six tubes of pure agar culture of virulent plague bacilli of 27 hours' growth, recently obtained from autopsy of a human case. The pig showed no visible reaction, kept a good appetite and was abandoned on 1 March, 1909.

Pig No. 3. Male, brother of the former, of the same weight and colour. It was also inoculated on 1 January, 1909, intraperitoneally with a pure culture of virulent plague bacilli (the same as administered to the previous pig). The dose consisted of two tubes. There was no sensible reaction, the animal continuing to eat well. It was also abandoned on 1 March, 1909.

Pig No. 4. Black, male, weighing about 12 kilograms. On 8 February, 1909, it ate the bodies of four rats (*M. norvegicus*), without heads; cut into pieces and mixed with maize meal and cold water. The rats had been spontaneously attacked by septicaemic plague, and showed an enormous quantity of bacilli in their organs. On the following day the pig ate another rat (*M. norvegicus*) in the same condition as the former ones and the viscera of a cat, spontaneously attacked, and which had died of bubonic plague. On the following day it swallowed the organs of a man who had rapidly succumbed to the bubonic plague with septicaemia, that is, an inguinal bubo, 200 grams of liver, the gall-bladder and 100 grams of peri-bubonic cellular tissue with strong haemorrhagic oedema. All the organs contained a large quantity of plague bacilli. The animal always kept in good health, being abandoned on 24 April, 1909.

Pig No. 5. Black, male, 13 kilograms in weight. It swallowed on 12 February, 1909, a pure plague culture of 48 hours' standing (corresponding to 14 agar tubes) mixed with maize meal and cold water¹.

¹ Judging from test guinea-pig inoculations the dose of virus swallowed by this pig was sufficient to kill, by subcutaneous injection, 1,400 guinea-pigs of 300 grams in weight.

Furthermore, on the following day, it ate all the body of a rat (*M. norvegicus*) spontaneously attacked and killed by septicaemic plague, with an enormous quantity of bacilli in all its organs. The pig gave no sign of illness, having considerably fattened until abandoned on 24 April, 1909.

Pig No. 6. Black, female, 12 kilograms in weight. It received in the peritoneal cavity on 14 February, 1909, the contents of 79 or 80 Petri dish cultures 48 hours old. The dishes (11 cm. in diameter) were sown with plague bacilli recently obtained from a human body; they were so virulent that an eightieth part of the quantity injected into the peritoneal cavity of the pig killed a guinea-pig weighing 370 grams in 3½ days. The same pig received subcutaneously, on the same occasion, under the same conditions already referred to, an agar culture (Petri dish) mixed with physiological salt solution. At the same time a square decimeter of the skin of the dorsum of the animal was scarified and rubbed strongly with the contents of another Petri dish. Finally the nasal mucous membrane was scarified and rubbed with pure plague culture of the same origin.

The pig did not fall sick. It left the Laboratory in a very fat condition on 24 April, 1909.

Pig No. 7. Black, male, 13 kilograms in weight. It ate on 15 February, 1909, mixed with maize meal, the following portions of the organs of a human case which had died on the same day of bubonic plague with septicaemia (after 36 hours' illness): bubo, 20 grams; spleen, 200 grams; liver, 200 grams; lung, 80 grams, sanguineous fluid, 50 grams. All these organs contained numerous plague bacilli¹.

We did not note any morbid alteration in this pig, in spite of most careful observations. It was abandoned in a much fatter condition on 24 April, 1909.

Pig No. 8. Black, female, 10 kilograms in weight. On 9 March, 1909, it swallowed, mixed with maize meal, pieces of organs from a case which had died on the same day of primary pneumonic plague, viz. all the lung tissues affected by hepatisation and weighing 1,000 grams; part of the spleen of the same, 60 grams; piece of the stomach with sanguineous suffusion, 50 grams. Smears from the lung appeared to be made up exclusively of plague bacilli. On the following day the pig again swallowed in the same vessel human plague organs, this time the

¹ This quantity of virus would be sufficient to kill by subcutaneous inoculation 8,800 guinea-pigs of 250 grams in weight each, as we calculated by the inoculation of some of these animals.

following parts: the lung of another case which had died of primary pneumonic plague, 600 grams; spleen of the same, 200 grams. There was also in the lung a large number of plague bacilli¹.

On the day following the second ingestion the pig appeared low-spirited and without appetite, but this indisposition only lasted some hours, after which the animal ate the afternoon meal very well and continued to keep up its good health until finally abandoned on 24 April, 1909.

Dogs.

Dog No. 1. Male pup, 1·5—2 months old, about two kilograms in weight, of no particular breed. On 4 January, 1909, it ingested a dose of pieces of spleen from a case which had died on the same day of septicaemic plague. In this spleen there was a very large quantity of plague bacilli².

The pup gave no signs of illness and was abandoned on 10 February, 1909.

Dog No. 2. A pet dog (*C. vellosus*), male, 700 grams in weight. On 4 January, 1909, it took *per os* portions of bubo and human spleen of a person who died on the same day of septicaemic plague and showing a very large number of plague bacilli. On this same occasion a portion of the same spleen was converted into an emulsion with salt solution which was injected subcutaneously by the flank³.

The animal died in less than 48 hours. The autopsy made a few hours after death showed the following lesions: at the site of inoculation an extensive zone, reddish-violet and very oedematous and mortified, the oedema extending to the lower corresponding member and to the opposite side of the abdomen; generalised congestion; there were no inguinal buboes, but on the neck there were two glands, one on each side, with sanguineous suffusions. Tonsils very hypertrophied and the lungs strongly congested; spleen dark, very succulent, liver very red and with yellowish zones of superficial necrosis. In a short portion of the

¹ The 1,600 grams of lung swallowed by this pig would have sufficed to kill *per nasum* and *per os* about 220,000 guinea-pigs of 250 grams in weight according to our calculations, after having inoculated a few.

² The dose of virus ingested by this dog would be sufficient to kill, by subcutaneous inoculation, 200 guinea-pigs of 250 grams in weight, as was calculated by injection in some of these animals.

³ The quantity of virus administered to this animal was sufficient to kill, by subcutaneous inoculation, 900 guinea-pigs of 300 grams in weight each, as we calculated by injection of some of these animals.

small intestine near the large one the intestinal mucous membrane was highly congested. The bacteriological examination of the smears from the different organs gave the following results: mortified zone of the site of inoculation, many microbes, plague-like ones being rare. Cervical glands: Some microbes not of plague-like appearance. Pelvic ganglions nil. Mesenteric glands nil. Tonsils: rare plague-like bacilli. Lungs nil. The cultures revealed a long bacillus, staining faintly, without the morphological characteristics of the typical plague bacilli, showing notwithstanding some of their reactions (chains in broth and involution forms in salt agar). With this bacillus we did not succeed in bringing about the death of the injected guinea-pigs. Mixed with this bacillus a staphylococcus, stained by Gram's method, existed in the culture but we did not try to identify it.

Dog No. 3. Male, pet dog, mongrel, weighing about 8 kilograms. On 4 January, 1909, an agar culture of pure plague bacilli was injected intraperitoneally¹. The animal showed no signs of illness, kept good appetite and was abandoned on 10 February, 1909.

Dog No. 4. Male, setter, weighing about 10 kilograms. On 1 February, 1909, we gave it, *per os*, the spleen and buboes of a cat spontaneously infected by plague, and a piece of spleen and a bubo from a case which succumbed on the same day from septicaemic plague of rapid evolution. These organs contained a large number of plague bacilli. There was no sign whatever of sickness, and the dog was abandoned on 15 February, 1909.

Dog No. 5. Male, lap-dog, mongrel, about 8 kilograms in weight. On 14 January, 1909, we shaved a portion of the skin of the back, about one square decimeter, which we scarified. On this surface we gave a hard friction with fragments of human organs full of plague bacilli and derived from a person who had rapidly succumbed to septicaemic plague. The dog showed no morbid sign, and the animal was abandoned on 15 February, 1909.

Dog No. 6. Male, mongrel, weighing about 15 kilograms. A cutaneous, parasitic disease produced many sore spots over all the skin of its back. This large ulcerated surface was taken advantage of to friction it with a human plague bubo which contained an enormous quantity of bacilli and which was derived from a person who had rapidly died from septicaemic plague. The dog kept up a good appetite, showing no signs whatever of illness, and was abandoned on 15 February, 1909.

¹ The dose injected would be sufficient to kill 400 guinea-pigs of 250 grams in weight each.

Rabbits.

Ten rabbits were inoculated. They were of different sizes, both wild and tame, and were subjected to cutaneous, subcutaneous, peritoneal and *per os* inoculations. For three of the inoculations we used organs of plague-infected human beings, rats and cats and also virulent cultures of plague bacilli. They all died within three to five days, a curious fact being that a tame animal of 2500 grms. in weight succumbed rapidly to a cutaneous friction over a small surface of two square decimeters with plague-infected organs.

Ferrets.

Ferret No. 1. Full-grown male. On 24 January, 1909, we injected subcutaneously a strong dose of virulent pure plague culture. The ferret died in less than four days, showing a necropsy appearance similar to the guinea-pigs and infected in the same way with numerous plague bacilli, as shown by inoculation in guinea-pigs.

Ferret No. 2. Full-grown male. Having shaved about four square centimetres of the dorsal skin, we frictioned it strongly on 18 January, 1909, with a human bubo full of plague bacilli and derived from an individual who had died rapidly from septicaemic plague. The ferret appeared to be healthy until 1 February, when it appeared run down, but still had an appetite. It died on 5 February. The autopsy showed the following lesions: a not very intense subcutaneous congestion, cervical right gland red and swollen, but without the surrounding tissues being inflamed; the left cervical gland, however, was the seat of a plague bubo. There were no noticeable crural or axillary glands; spleen very large; liver dark, smooth, and very congested. Congestion of the lungs, a retro-peritoneal suppurated gland, a mesenteric gland with the characters of the secondary plague bubo of the Austrian Commission; acute supphritis with fatty degeneration. The examination of smears showed the following results: blood: negative. Right cervical gland: numerous plague-like bacilli. Retro-peritoneal gland: negative. Mesenteric gland: few plague-like bacilli. In the cultures no bacillus was obtained that showed plague characteristics. The inoculations also did not reveal infection by plague.

Cats.

Cats Nos. 1 and 2. Both full-grown males. On 21 February, 1909, they ate small pieces of human bubo, spleen and liver highly infected with plague bacilli and derived from an individual who had died in three

days of bubonic septicaemic plague. The cats, three days after the infection, appeared ill and without appetite. It was afterwards noticed that the necks of both appeared swollen, above all in the sub-maxillary regions. On 5 March one of the cats died. At autopsy exactly similar appearances were observed to those of many other spontaneously infected cats we had occasion to examine on Terceira Island. Slightly generalised subcutaneous congestion, swollen glands in all parts, suppurating cervical buboes on both sides, including the sub-maxillary and carotidian glands, the left bubo being the larger, necrotic amygdalitis with large oedema of fauces, tracheitis, broncho-pneumonia with miliary nodules; spleen large and of a claret colour; liver very granular and congested; intense conjunctivitis and keratitis with hypopion. The organs of this animal showed: buboes: numerous plague-like bacilli. Ocular pus: some plague-like bacilli and phagocytes infected with degenerated bacilli. Liver and spleen: few plague-like bacilli. Kidneys: few plague-like bacilli. Lungs: numerous plague-like bacilli. The cultures of the spleen, liver, lungs, blood and bubo revealed plague bacilli. These were determined by inoculation tests.

The second cat was seriously ill for three weeks, but recovered and to-day belongs to one of us.

Goat.

Young female of 8 kilograms in weight. On 12 February, 1909, it received a pure and virulent plague culture of seven agar tubes emulsified in physiological salt solution; two-thirds were introduced intraperitoneally and the remainder subcutaneously. The cultures were taken from human corpses and from that of a cat. The kid gave no signs of acute illness, but it was noticed that it grew thin, and on 1 March, 1909, it would not feed. On the following day it became blind and knocked its head against the walls, dying on 4 March, 1909. The autopsy showed the axillary, inguinal, cervical and popliteal glands to be succulent, but white; the mesenteric and retro-peritoneal glands had the same characteristics. Spleen small and dry. Liver smooth and congested. Lungs discoloured and oedematous. Smears taken from all the glands referred to, and also from the spleen, liver, lungs, kidneys and blood, showed no bacilli at all. All the cultures made from the same organs proved sterile, excepting one from the pelvic gland that showed a bacillus unlike that of plague.

Pigeons.

On 5 January, 1909, eight pigeons were inoculated with cultures of pure and virulent plague bacilli and with the organs of spontaneously plague-infected rats containing a large number of bacilli. We made use of the subcutaneous, intraperitoneal and *per os* inoculations, large doses being administered to each bird¹. None of them showed any sign of illness, and the experiments were abandoned on 1 March, 1909.

Turkey.

Full-grown male. On 12 February, 1909, we forced it to swallow the viscera (spleen, liver, kidneys, supra-renal capsules and lung) and the buboes of three spontaneously plague-infected rats that had succumbed to this disease and in which there was a very large number of bacilli. The turkey at no time showed the slightest sign of illness, keeping up a good appetite until it was abandoned on 24 April, 1909.

Ducks.

On 12 January, 1909, three ducks were inoculated (two of them being males and one female), one subcutaneously, another intraperitoneally and the third *per os*, with the organs of a guinea-pig inoculated with plague, to which it had rapidly succumbed, and of a spontaneously infected rat. The organs contained a large number of plague bacilli². The ducks did not suffer at all, and continued to eat well until they were abandoned on 24 April, 1909.

Chickens.

1st Series. On 20 December, 1908, we inoculated three chickens, two hens and one cock of 900, 910 and 950 grams respectively, with the livers and spleens of spontaneously plague-infected rats with numerous bacilli in these organs. To one fowl we administered *per os* one-third

¹ The dose of culture administered to each animal would be sufficient to kill 50 guinea-pigs of 300 grams each, and the dose of plague-infected organs that fell to the lot of each one would be sufficient to kill 60 guinea-pigs of the same weight, as we calculated by inoculation experiments on some of these animals.

² The dose of mixture of organs administered to each duck would be sufficient to kill, by subcutaneous inoculation, 300 guinea-pigs of 330 grams each in weight, according to experiments made on some of these animals.

of the material employed, with which we also frictioned the nostrils and the nasal fossus. One-third of the material was introduced, emulsified in physiological salt solution, under the skin of the second fowl, and the remaining third was introduced in the same way into the peritoneal cavity of the third bird¹.

Only the bird inoculated intraperitoneally fell sick, showing in the rectum a temperature of 42.5° C. which was maintained for two days, during which it ate little, and had a down-cast appearance with the feathers standing up. It rapidly got better and was abandoned with the others on 24 April, 1909.

2nd Series. On 25 December, 1908, we inoculated 12 chickens, 11 full-grown and a cock-chick. We mixed with cold maize meal virulent plague cultures of human origin which we made the birds swallow, also frictioning their nostrils with the same material. The 11 chickens did not suffer at all, whilst the cock-chick died some days after through the bad treatment inflicted on it by the other chickens (cranial wounds). The autopsy of this chick revealed no signs whatever of infection. The examination of the smears from its organs was negative, the cultures sterile and the inoculations unsuccessful. The 11 chickens were abandoned on 24 April, 1909.

3rd Series. On 15 February, 1909, 25 grown-up chickens were inoculated intraperitoneally and *per os* with pure and virulent plague cultures obtained from the autopsy of an individual who died from a rapid form of illness².

Of these 25 birds, 24 without doubt did not suffer at all, and were abandoned on 24 April, 1909. We ought to note that we placed some Terceira Island guinea-pigs and rabbits (which we have seen are very susceptible to plague) along with the fowls. In spite of close contact with the faeces of the chickens, none of these animals showed any sign of illness. Some days after the inoculation of these 25 chickens (according to our habit, the birds remained under observation in the Laboratory for at least 10 days) another chicken that was under observation in another place escaped, and through the carelessness of a servant it got in among the 25 chickens. Eight days later one of the 26 chickens died, unexpectedly, as it had eaten well at the morning

¹ The quantity of virus administered to each bird would be sufficient to kill 400 guinea-pigs of 250 grams, according to experiments made on some of these animals.

² The dose inoculated into the peritoneal cavity of each bird would be sufficient to kill 40 guinea-pigs of 250 grams each, and that administered *per os* enough to kill 800 guinea-pigs of the same weight, as we calculated by inoculating some of these animals.

meal. We were in doubt whether the chicken which died was the intruder or not. The chicken did not however die of the plague, as was proved at autopsy when the following lesions were observed: severe pulmonary congestion, enteritis with blood suffusions in the intestinal mucous membrane, the contents of which had some modified blood; peritonitis, with haemorrhagic fluid and agglutination of the intestines by false membranes. Smears of the lungs and contents of intestines showed plague-like bacilli, but the cultures did not give chains in broth nor Hankin's involution forms in salt agar. Inoculations into guinea-pigs caused the death of these animals in less than 24 hours, but plague bacilli could not be detected. The disease was not chicken-cholera, but seemed to us to be what French veterinary surgeons call infectious enteritis of chickens.

4th Series. On 12 March, 1909, we inoculated 25 full-grown chickens, intraperitoneally and *per os*, but with a dose more or less double to that administered to the other series. These birds showed no sign of illness, and were abandoned on 24 April, 1909.

5th Series. On 15 March, 1909, we forced 20 full-grown chickens to swallow pieces of a human lung—primary pneumonic plague with lobar hepatisation and with an enormous quantity of plague bacilli¹.

None of these birds showed any sign of illness, and were abandoned on 24 April, 1909.

CONCLUSIONS.

(1) Although our experiments on calves may not be extensive, we think we may affirm that the bovine race, in spite of the large doses inoculated, did not contract the plague.

On Terceira Island we had no knowledge whatever of any case of illness in bovines which could be put down to plague.

(2) As for the pigs, we consider the series of animals on which we experimented fairly large. The doses of virus inoculated were really enormous, far larger than what they would naturally receive.

The experiments led us to conclude that pigs do not contract plague².

¹ The dose ingested by each bird would be sufficient to kill by subcutaneous inoculation 1,000 guinea-pigs of 250 grams each, according to the calculation made by inoculation of some of these animals.

² We succeeded in examining the corpses of four pigs that died rapidly of infectious diseases on Terceira Island. In two of them we noticed illnesses which cause reddish spots on the skin, with haemorrhagic enteritis and generalised adenitis. We ought to state

(3) The experiments made on dogs seem to show that only with very large doses of plague bacilli can infection of these animals be obtained, and it seems beyond doubt that in its normal condition the dog is an animal practically refractory to the plague.

This conclusion proved very interesting to Terceira Island, where dogs have rendered and continue to render great service in rat hunting¹.

(4) The experiments made on rabbits led us to conclude that this animal is, on Terceira Island, very susceptible to plague.

(5) Our experiments on ferrets were made because these animals are extensively employed in rat-hunting, above all in the country.

According to these experiments the ferret is an animal susceptible to plague, but only able to contract an acute form of this disease by inoculation of large doses. However, as the ferret sucks the blood of the rats its use in hunting these animals is not recommended².

(6) Our experiments corroborate those of the Austrian Commission that cats can be infected by plague *per os*, after which they show autopsy

that the adenitis did not appear at all like primary plague buboes of the Austrian Commission, and that the intense reaction so characteristic of the neighbouring glandular tissues was missing. These two pigs died of a pasteurellosis that, in our opinion, can never be taken for the plague by anyone accustomed to see this disease. This conclusion is founded on the bacteriological examination of smears from organs, cultures and inoculations in guinea-pigs. Two other pigs succumbed to infection, the lesions in the fauces, epiglottis, larynx, trachea, bronchial tubes and lungs being especially pronounced, there being even false membranes and ulcerations in the larynx, besides sub-mucous congestion and sub-mucous oedema.

In these pigs there were also glandular enfarcts that were far from resembling, however, plague buboes (whether primary of the first or second order, or secondary). There was no haemorrhagic septicaemia in these two pigs; it was probably, however, the illness which French veterinary surgeons call swine pneumo-enteritis, a conclusion drawn from the character of the isolated bacillus and the effect of their inoculation into experimental animals.

We may mention that one of these two latter pigs was under observation in the Laboratory where it was destined to serve for inoculation experiments with plague.

These observations strengthen the conclusions about the insusceptibility of pigs to plague.

¹ We examined the bodies of two dogs, which had rapidly died of infectious diseases. We did not certify the existence of plague, either by anatomo-pathological signs or by bacteriological examination. We can equally mention that we had no knowledge of any case whatever of illness in these animals that could bear any relation to the epidemic.

² We examined the bodies of three ferrets spontaneously infected on Terceira Island. Two of them had suppurating cervical glands in which we did not succeed in identifying the plague bacillus; but the third, having entered a rat-hole, killed one of these animals and died from plague, as was verified by us.

appearances very similar to those of cats spontaneously infected by plague with buboes in the neck¹.

(7) The general conclusion which we draw from our experiments on four kinds of birds, namely, pigeons, ducks, turkey and chickens, especially in regard to the latter, is that these animals are insusceptible to plague².

¹ We made a fairly long analysis of cat-epizootic plague on Terceira Island, finding 23% (23 out of 100 cats examined) attacked by plague. The majority of these cats had cervical buboes, some at least had probably been infected *per os*; others had axillary, inguinal and popliteal (only one) buboes; and in these cases the infection probably occurred through the agency of fleas. Finally, two succumbed to primary pneumonic plague. We will enter more into detail on this subject in a future paper.

² On Terceira Island we examined a considerable number of birds which had spontaneously died during the plague epidemic, namely, 1 parrot, 3 turkeys, 1 sea-gull, 1 blackbird, 4 pigeons and 17 chickens. None of these birds showed any signs of plague on the bacteriological and post-mortem examinations. One of the pigeons was a victim to an acute infectious disease which appeared to be due to an unidentified coccus. Some chickens died of chicken-cholera, and it should be noted that the lesions observed at autopsy could not be confounded with those of plague-infected animals, the bubo being absent, whether the primary of the first or second order, or the secondary of the Austrian Commission.

We are convinced that a sufficiently long practice of the pathological anatomy of plague does not allow us to confound the lesions of this disease with those of chicken-cholera.

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OBSERVATIONS ON LEUCOPROTEASE AND “ANTI-LEUCOPROTEASE.”

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2 Figures.

ACCORDING to Kossel (1888) Fritz Müller discovered that phthisical sputum digests fibrin and coagulated albumen in weakly alkaline media, but the fact that antiseptic pus digests proteins seems to have been first observed by Leber.

Achalme (1899) separated proteolytic ferments from pus from various sources and Erben (1903) and Schumm (1904) drew attention to the notable quantity of albumoses in blood from cases of myelogenous leukaemia which had been incubated, and the relative absence of this proteolysis in the incubated blood from cases of lymphatic leukaemia. Ascoli and Mareschi (1901) showed that the peritoneal exudate produced by aleuronat underwent autolysis under aseptic conditions.

More recently the subject of a proteolytic ferment in leucocytes has been studied by a number of authors amongst which the principal are Opie (1905 and 1906), Müller and Jochmann (1906 I. and II.), Jochmann and Lockermann (1908), and Fiessinger and Marie (1909).

All agree as to the presence of a tryptic ferment in the polynuclears but the existence of another protease acting in feebly acid solutions in lymphocytes described by Opie is questioned by Müller and Jochmann and Fiessinger and Marie.

Opie working with sterile exudates obtained by injection of aleuronat into pleural cavities of dogs, as well as with pus from turpentine-produced abscesses in various animals, found that in the leucocytes collected from such artificially produced exudates by centrifugalisation two ferments may be demonstrated. The main point of the proof of the separateness of these ferments is that in an alcohol ether dried preparation only one remains. He calls the two ferments *lymphoprotease* and *leucoprotease*.

Lymphoprotease is associated with mononuclear leucocytes and resembles pepsin in acting at low degrees of acidity but it is not pepsin as it fails to act in 0.2 % HCl though it will do so in feebler dilutions. It is inhibited by alkalinity but not by the serum as such.

Leucoprotease is associated with the polymorpho-nucleated cells and is a tryptic-like ferment acting best in faintly alkaline but well also in neutral media; Opie does not think this is trypsin for it is very weak compared with the latter. It is held in check by normal sera. It is not destroyed by treatment with alcohol and ether and drying. The various results of Opie and others with regard to other points will be found in their respective sections.

Lymphoprotease was also obtained by Opie and Barker (1907) by injecting tubercle bacilli into the pleural cavity of dogs. It has been previously indicated that its origin from lymphocytes has been disputed but as my own experiments deal only with leucoprotease from human pus the question will not be entered into.

I was anxious to inquire into certain points as regards the working of the so-called "anti-body" manifested in normal sera, and especially to see if one could increase this anti-proteolytic power by immunisation and if there could be found any evidence in favour of or against the idea that any anti-effect is due to a true anti-body similar to those concerned in production of immunity to various toxins and so forth. This work is not intended to be complete—the subject is in itself rather complicated and it has been difficult for me even with such facilities as I have had at the Lister Institute to get sufficient material—hence I have merely been able to cover part of the ground, the remainder of which I hope to attempt on a future occasion. Two difficulties which I have found to render accurate work on certain points so far almost impossible are, firstly the ferment in my preparations is very weak, and secondly it is always accompanied by large amounts of proteid, and I have not so far been able to devise a satisfactory method, that is to say necessarily a conservative method, by which I can at all purify the preparation.

Methods.

The material I have examined was derived from thirteen specimens of empyema pus. In the majority of experiments the material was, after screening off as much blood clot, fibrin, etc. as possible, centrifuged and washed with normal saline three or four times. The final debris was shaken well with methylated spirit, this either filtered or centrifuged, and pipetted off and then absolute alcohol and finally alcohol-free dried ether used in the same way. The debris from this process was collected in flat trays, fanned vigorously for a while and powdered, then placed in a desiccator, till apparently completely dry, if necessary re-powdered, and then kept in the desiccator till required. Here apparently it is little affected as regards its activity by lapse of time. The final product is a very fine dry powder which has a colour varying from white to pink, according to the amount of blood clot present.

For estimating the activity of the enzyme the following process, which is similar to that used by Hedin in his experiments with trypsin, was employed. This only differs in detail and the form of precipitation of the proteid from that of Opie. Measured amounts of ferment preparation, either by weighing quantities dried of the powder or pipetting suspensions or extracts, were taken into suitable stoppered bottles of about 150 c.c. capacity. To this was added the substrate used, either 25 % boiled solution of casein with toluene, or boiled fibrin, or gelatin, or egg albumen. In testing the effect of serum this was also added. Controls were made with the enzyme preparation heated in moist condition above 90° C. for several minutes. To each flask a definite amount of toluene was added as preservative and the flasks well shaken and incubated in a hot room, where they were kept in motion by an electric shaker. At the end of the digestion period the flasks were taken off, cooled to the room temperature, and precipitated by equal amounts of the tannic acid solution. The mixture was well shaken and filtered. The filter papers should be all of one kind and the same size, as also should be the filter funnels. The temperature at which the precipitation was carried out seemed to be important, as also did the time the materials were left in contact before filtering. A feature of interest noticed was that a perfectly clear filtrate, which subsequent estimation proved to contain large amounts of proteid, usually shows after some minutes a faint cloudiness, very hard to separate. This does not occur in the control flasks and the amount of proteid in

this precipitate is negligible, as whether one refilters to clearness or not, the results were within my experimental error.

Aliquot parts of these filtrates were Kjeldahled in the usual way and the ammonia estimated. The index of activity is furnished by the amount of ammonia in the distillates. The figures are expressed as ammonia represented by c.c. N/10 acid.

The potency of my preparations was not great and the following two experiments which are given in detail illustrate the order of activity obtained.

Powder No. 4. Time of digestion 5 days. Substrate casein 2.5 % in 0.25 % sodium carbonate solution.

		Nitrogen as c.c. of deci-normal sulph. acid in 100 c.c. of filtrate
A.	Substrate 100 c.c. + powder 0.04 gm.	= 16.9
B.	Substrate 100 c.c. + powder 0.04 gm. (heated)	= 4.1

Each flask precipitated with 100 c.c. tannic acid solution. 100 c.c. of filtrate taken for estimation.

Powder No. 6. Time of digestion 5 days. Substrate casein 2.5 % in 0.25 % sodium carbonate solution.

		Nitrogen as c.c. of deci-normal sulph. acid in 50 c.c. of filtrate
A.	Substrate 50 c.c. + powder 0.4 gm.	= 30.6
B.	Substrate 50 c.c. + powder 0.4 gm. (heated)	= 3.0

Each flask precipitated with 50 c.c. tannic acid solution. 50 c.c. of filtrate taken for estimation.

Influence of Amount of Enzyme upon the Rate of Action.

The following experiments were undertaken with a view to inquire into the rate of change brought about by the enzyme in the alcohol ether dried preparation. Bottles containing varying amounts of the powder with equal amounts of substrate were allowed to digest for varying lengths of time. In the first chart the abscissa represents time in days and the vertical ordinates thereto activity measured as cubic centimetres of deci-normal sulphuric acid. There are four curves of which the lowest represents the results obtained by allowing 0.05 gram of the powder to act on equal amounts of substrate for varying times. Four bottles containing identical amounts of substrate and powder were put into the hot room at the same time. One of these was taken out on the expiration of the times mentioned in the chart and the nitrogen as ammonia in the tannic acid filtrate estimated. The other three curves similarly represent activity at varying times of 0.1 gram, 0.2 gram and

0.4 gram respectively. They were obtained in the same way except that the curve for 0.2 gram was not continued so long and the estimations were made at different times.

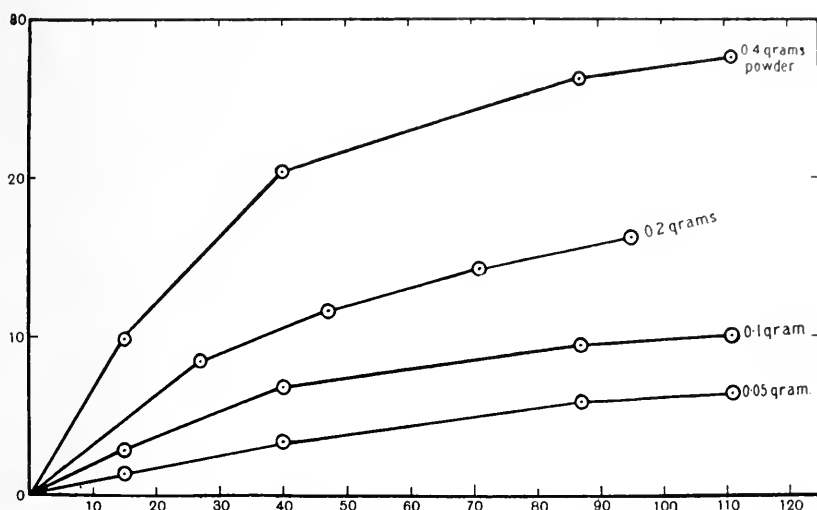


Chart 1.

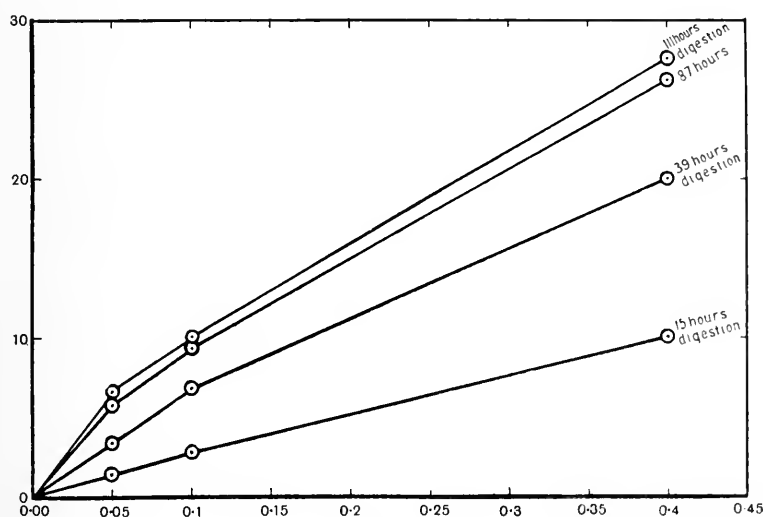


Chart 2.

The second chart is compiled from the same figures and shows certain points more clearly. In it the abscissa represents the amounts of enzyme while the vertical ordinates represent activity as cubic centimetres of deci-normal acid representing ammonia in filtrate. There are four curves at 15, 39, 87, 111 hours respectively showing effect of increasing amounts of enzyme.

The figures following the charts are the protocols of experiments from which the charts are compiled.

Experiment 5.

Powder No. 6. Time $2\frac{1}{2}$ days. Substrate, casein 2.5 % sol. in sodium carbonate solution 0.25 %.

Powder		Casein sol.	Time		Nitrogen as c.c. of deci-normal sulph. acid in 50 c.c. of filtrate
0.05 gm.	+	50 c.c.	15 hrs.	=	4.4
0.05	+	50	39	=	6.4
0.05	+	50	87	=	8.8
0.05	+	50	111	=	9.4
0.05 (heated)	+	50	41	=	3.0
0.1 gm.	+	50 c.c.	15 hrs.	=	5.9
0.1	+	50	39	=	9.8
0.1	+	50	87	=	12.5
0.1	+	50	111	=	13.0
0.1 (heated)	+	50	111	=	3.0
0.2 gm.	+	50 c.c.	27 hrs.	=	11.6
0.2	+	50	47	=	14.5
0.2	+	50	71	=	17.1
0.2	+	50	95	=	19.2
0.2 (heated)	+	50	47	=	3.0
0.4 gm.	+	50 c.c.	15 hrs.	=	12.9
0.4	+	50	39	=	23.2
0.4	+	50	87	=	29.3
0.4	+	50	111	=	30.6
0.4 (heated)	+	50	111	=	? about 3

As precipitant 50 c.c. tannic acid sol. was used, 50 c.c. of the filtrate after this addition was taken in each case. The flasks were well shaken during the first ten hours and for short spells afterwards.

The points brought out by the above results with regard to the action of the enzyme under the described conditions, *i.e.* acting in closed flasks on a large excess of proteid, may be summarised as follows:

Influence of amount of enzyme time constant. Chart 1.

1. There is an increase in the total action with the time up to the point of termination in these experiments. From the charts it seems that the maximum was nearly but not quite reached.

2. The amount of change per unit time is relatively greater in the earlier hours, falling off with the progress of time.

3. In the early hours and with small doses the rate of change is nearly proportional to the time.

Influence of amount of enzyme time constant. Chart 2.

1. The total action increases with the amount of enzyme (even the largest dose in these experiments is small relatively to the total proteid to be changed, as similar experiments with 0.8 gram showed a great increase of action).

2. With amounts of enzyme up to 0.1 gram per 50 c.c. and digestion time not exceeding 39 hours the rate of hydrolysis is proportional to enzyme added.

3. On increasing the amount of enzyme or the interval for digestion the rate falls more and more short of direct proportionality.

Effect of Reaction.

The following experiments show the effect of reaction on the activity of the powder.

Experiment 6.

Powder No. 3. Substrate, fibrin 0.5 gm.

		Nitrogen as c.c. of deci-normal sulph. acid in 20 c.c. of filtrate	
A.	Boiled fibrin 0.5 gm. + powder 0.02 gm. + acetic acid 0.2 % 15 c.c. ...	=	0.8
B.	Boiled fibrin 0.5 gm. + powder 0.02 gm. + sodium carbonate sol. 0.25 % 15 c.c. ...	=	2.0
C.	Boiled fibrin 0.5 gm. + powder 0.02 gm. + water 15 c.c. ...	=	1.15
D.	Boiled fibrin 0.5 gm. + powder 0.02 gm. } (boiled) + water 15 c.c. }	=	0.5

Added 15 c.c. tannic acid solution. Took 2 c.c. of filtrate for estimation.

This experiment displays the absence of action in weakly acid media while there is action in approximately neutral or alkaline media.

The following experiment conducted by a different method also gives us the same results. Here the experiment is merely qualitative. Small and equal quantities of the powder were allowed to act on gelatin at incubator temperature (37° C.) under the conditions of reactions shown, and examined as to power of solidifying by cooling in running water.

Experiment 7.

Powder No. 4a was used and toluene as antiseptic. These were added to a large test tube with $2\frac{1}{2}$ inches of 10 % gelatin.

Reaction	14 hrs.	38 hrs.
Just alkaline to Lachmoid	fluid	fluid
Just acid to Lachmoid	solid	solid
Neutral to Lachmoid	fluid	fluid

Another qualitative experiment shows the same results. Here the method employed as a test of activity was the biuret test. Equal amounts of the powder No. 6 were allowed to act on boiled dried fibrin powder in the presence of acid alkali and neutral diluents. After 24 hours they were examined with the following results:

Experiment 8.

	Biuret reaction	
1. Powder + fibrin + acetic acid 0.2 %	—	
2. Fibrin + acetic acid 0.2 %	—	
3. Powder + fibrin + sod. carb. sol. 0.25 %	+	+
4. Fibrin + sod. carb. sol. 0.25 %	—	
5. Powder + fibrin + normal saline	+	+
6. Fibrin + normal saline	—	

N.B. A solution of the powder itself does not give the reaction.

The above results show that the enzyme contained in the dried preparation from human pus acts best in weak alkaline solutions, also well in approximately neutral solutions but not in the presence of 0.20 % acetic acid.

Effect of Heat.

The following experiments were performed to show the effect of heat on the enzyme, *i.e.* to determine the point at which the enzymotic activity is no longer manifested. On investigation it becomes apparent in all such research that a factor equally important to the temperature to which the substance is raised, is the time taken to do so. It is not long before one realises the futility of saying that the death point is such and such a temperature. This will be found to differ considerably with the method used. If for instance the dried powder is simply heated in that dry condition in a hot air stove, it will resist for many minutes, up to a quarter of an hour at least, a temperature well over 100° C. Whereas,

f the powder is moistened, it is killed at a far lower point; but besides this the time taken in heating is very important. Conditions are entirely different if we start heating the ferment from room temperature and bring it up to the required point than if we drop the ferment into a fluid heated to that point. Likewise if we keep the ferment less or greater time at any point so the result will differ. Two methods which I have tried give results showing what I mean, in Experiment 9 the heating was done in similar test tubes in a water bath already heated to the required amount. In this series the ferment is markedly affected by one minute at 65° to 67° C., that is to say one minute after the ferment reached 65° C. In Experiment 10, where the ferment is merely dropped into the water when that has reached the required temperature and is retained there the requisite time, it is not much affected by one minute between 69° and 71° C.

Other considerations, too, render this question I think one which gives very unprofitable results unless some standard method be adopted by all observers. Opie (1906 (1)) gives 67° C. as the thermal destruction point; but unfortunately gives little information as to his method. When it is an obvious fact that such factors as amount of proteid in solution, rate of rise of temperature, and others such as are stated above, do affect the results, it is hardly likely that any two observers will agree. Here I merely give the results of a few experiments which were really done as a means to know how I could most effectively kill the enzyme or controls.

Experiment 9.

Method. The heating was done by dropping weighed amounts of powder No. 9 into 10 c.c. of water raised to the required temperature in a water bath, taken off as required and cooled quickly. The 20 c.c. of water were heated in the bottles used for digestion and subsequently 50 c.c. of casein solution were added with 5 c.c. of toluene to each flask. After two days' digestion 50 c.c. of tannic acid solution were added and 50 c.c. of the filtrate from this taken for estimation. The figures represent nitrogen as deci-normal sulph. acid in 50 c.c. of the filtrate.

Heated to 62° C. and cooled immediately = 12·8.

„	65° C.	„	„	= 12·8.
„	70° C.	„	„	= 12·7.
„	75° C.	„	„	= 10·9.
„	80° C.	„	„	= 6·7.

Heated for one minute between 69 — 71° C. = 12·2.

„	„	„	69·5—71·5° C.	= 12·15.
„	„	„	72 — 73° C.	= 11·55.
„	„	„	74·5—75° C.	= 6·45.

Experiment 10.

Method. 0.2 grams of powder No. 5 were heated in 0.25 % sod. carb. sol. to various temperatures and kept at these temperatures for one minute. Then to each flask 20 c.c. of 10 % gelatin were added and the flasks put in the hot room (37° C.) to digest. Observations were made as to digestion by cooling the flasks under the water tap for the same time and noting the state of the gelatin.

	After 4 days	After 6 days
Powder not heated	fluid	fluid
1 minute at 65—67° C.	semi-solid	fluid
$\frac{1}{2}$ minute at 70—73° C.	solid	solid
1 minute at 73—75° C.	solid	solid
1 minute at 84—85° C.	solid	solid

Experiment 11.

Method. Powder was heated *dry* in watch glasses in a hot water jacketed stove to a temperature varying between 88—92° C. Then 0.1 gm. was allowed to act for three days on 15 c.c. 10 % gelatin with 15 c.c. sod. carb. sol. 0.25 %.

	Time left in stove	Nitrogen as deci-normal acid 20 c.c. of filtrate
A.	0	2.9
B.	5 minutes	3.0
C.	10 „	3.1
D.	15 „	3.3
E.	20 „	3.2
Control heated at 110—120° C. for several minutes in hot air stove		1.3

40 c.c. tannic acid added. 20 c.c. of filtrate used for estimation.

The Anti-Effect of Sera.

The anti-tryptic action of normal serum was noted by Hahn (1897) and Achalme (1901). Achalme succeeded in increasing the anti-tryptic effect of guinea-pig serum by immunisation. Hedin (1905 and 1906 I.) has very fully investigated the neutralising action of normal serum upon trypsin. This effect he finds is larger if the serum be added to the trypsin before adding the substrate and there is an increase in anti-action, up to a certain point, with the time for which they are left together before adding the substrate. The combination occurs more readily the higher the temperature, up to a certain point. The amount of trypsin neutralised is independent of dilution. The anti-power of the serum can be completely neutralised, but trypsin cannot be completely rendered inert by serum. The amount of trypsin neutralised is greater

relatively for small amounts than for large amounts of serum. The anti-body is completely destroyed by 0.1 to 0.2 % of acetic acid at 7° C. for 8 hours and is markedly affected by acid in a very short time. He found it impossible to separate anti-body and enzyme in a neutral mixture, *i.e.* to free the trypsin. In a further paper Eedin (1906 II.) compared the results obtained with serum with those obtained by the addition of charcoal to a tryptic digest. He found that this substance had a powerful effect in neutralising trypsin and that the neutralisation probably consisted of two consecutive stages. Firstly, a taking up or absorption of trypsin: in this stage the trypsin can be freed by the addition of more substrate. Secondly, a stage in which the trypsin is fixed. The amount fixed is larger the more charcoal used, the higher the temperature and the longer the time of interaction. He found this effect of charcoal to agree in every point as far as he could see with the effect of serum and concluded the latter to be a similar phenomenon.

The anti-tryptic action of normal sera was found by Landsteiner (1900) to be associated with the albumin fraction of the serum (Pick). This was confirmed by Cathcart (1904). Opie (1905, 1906) and Opie and Barker (1907), working with leucoprotease, found that serum either from purulent exudates or from blood inhibits the proteolytic effect of leucoprotease. This property is destroyed by acids and by heating to 75° C. for half an hour. A certain amount of serum is only capable of controlling a limited amount of enzyme. As in the case of trypsin the inhibiting action was associated with the albumin fraction of the serum and not with the globulin.

Müller and Jochmann (1906) also made observations on the anti-effect of serum. The anti-leucoprotease is not specific. The serum of one animal is equally effective against leucoprotease from all sources (Opie and Barker 1907).

The anti-body in normal sera neutralises both trypsin and leucoprotease (Jochmann and Lockermann 1908).

Variations in the anti-tryptic power of human serum have been found to occur in patients suffering from diseases involving cell destruction (Bittorf 1907) or associated with leucocytosis (Wiens 1907), tuberculosis (Wiens) and cancer (Brieger and Trebing 1908). Eisner (1909), however, finds that it is only in the cachectic state of cancer that the anti-tryptic power is modified.

In this country the subject has been exploited with a view to its possible utility in diagnosis by Golla (1909), Hort (1909) and Bayly

(1909). Golla has introduced means of considerable accuracy for measuring the anti-tryptic power of different sera under clinical conditions.

Before detailing my experiments with sera it will be useful to say a few words as to methods.

So as to make the total amount of proteid equal in each case, it was necessary in performing experiments with different amounts of fresh serum to make up the total serum content to the same figure by the addition of heated serum. Heated serum must also be used for maximum and minimum control. By suitable dilution we can arrive at a satisfactory method of getting a serum sufficiently heated and still in a liquid state. For this purpose I have found the dilution of 1 serum to 4 water the best, 1 serum to 4 normal saline is likely at times to precipitate before a sufficient temperature for use in controls is reached. For heating purposes I used a water bath already boiling; in this the dilute serum is immersed in a thin flask and kept moving briskly until the temperature as registered by a thermometer inside the flask reaches the required point. I have employed 90° C. as a useful temperature for heating all controls from the experience of such an experiment as No. 12. With regard to the effect of heat on the anti-power of serum, Opie found that heating equal quantities of serum diluted with equal amounts of salt solution to 75° C. for half an hour sufficed to destroy the anti-power. Lower temperatures seemed to afford a slight increase in the anti-power; he does not state how the heating was done. In the following experiment equal quantities, 10 c.c. of serum in the dilution of 1 serum to 4 water, were heated in the water bath from the water supply temperature to the required temperature in test tubes of equal thickness and calibre. Thus the time taken in heating to the required temperature should be in each case the same. The test tubes were immediately cooled under the water tap. This heated serum was allowed to act on a mixture of enzyme and substrate. In this case a fluid preparation $\frac{1}{2}\%$ of the powder in 0.25 % sod. carb. sol. was used in a dose of 20 c.c. in each flask. Of the casein substrate 50 c.c. were used in each flask. After $2\frac{1}{2}$ days' incubation 50 c.c. of tannic acid solution were added as precipitant and 50 c.c. of the filtrate from this taken for estimation. The results below show that it has begun to be affected at 72° C. and apparently is not much more affected by 76° C. But at 90° C. for several minutes it is completely destroyed so that this is a sufficient temperature to heat the serum for the controls.

Experiment 12.

Powder No. 5.					Nitrogen as deci-normal acid in 50 c.c. of filtrate
With fresh serum	3.05
With serum heated to 50° C.	3.15
" " " 55	3.2
" " " 60	3.0
" " " 70	3.2
" " " 72	4.30
" " " 74	4.25
" " " 76	4.25
Control at 90° C. for several minutes	4.95
Control with enzyme sol. heated to 90° C. for several minutes	1.95

The following experiments show the anti-power of the normal sera of ox and goats for leucoprotease.

Experiment 13.

Powder No. 5. Time 7 days. Ox serum collected two days before and kept on ice.					Nitrogen as deci-normal acid in 100 c.c. of filtrate
1. Powder 0.2 gm. + casein sol. 50 c.c.					
+ serum (1 to saline 2) 50 c.c. (heated)			=		36.6
3. Powder 0.2 gm. + casein sol. 50 c.c.					
+ serum (1 to saline 2) 50 c.c. (fresh)			=		23.8
5. Powder 0.2 gm. heated + casein sol. 50 c.c.					
+ serum (1 to saline 2) 50 c.c. (heated)			=		6.4

Heated in water bath to dryness from wet state. 100 c.c. tannic acid added. Took 100 c.c. filtrate.

Experiment 14.

Powder No. 5. Time 4 days under sol. 5 c.c. Serum Ox collected previous day. Dil. serum 1, normal saline 3, a total of 50 c.c. of diluted serum in each. The heated serum was raised to 91° C. Substrate, casein sol. 2.5 % in 0.25 % sod. carb. sol.

Powder		Casein sol.		Dilute serum			Nitrogen as deci-normal acid in 100 c.c. of filtrate
				Heated	Unheated		
1) 0.2 gm.	+	50 c.c.	+	50 c.c.	0 c.c.	=	37.0
2) 0.2	+	50	+	40	10	=	29.0
3) 0.2	+	50	+	25	25	=	22.3
4) 0.2	+	50	+	0	50	=	18.0
5) 0.2	+	50	+	50	0	=	5.1 control

Tannic acid 100 c.c. 100 c.c. of filtrate taken for estimation.

Experiment 15.

Same conditions with same serum. Time 2 days.

Powder		Casein sol.		Dilute serum			Nitrogen as deci-normal acid in 100 c.c. of filtrate
				Heated	Unheated		
1) 0.2 gm.	+	50 c.c.	+	50 c.c.	0 c.c.	=	26.1
2) 0.2	+	50	+	25	25	=	16.3
3) 0.2	+	50	+	0	50	=	11.0

Tannic acid 100 c.c. 100 c.c. of filtrate taken for estimation.

Experiment 16.

Powder No. 6. Time $2\frac{1}{2}$ days. Substrate, casein sol. 50 c.c. Goat serum, diluted, serum 1, normal saline 4. Total of 20 c.c. serum in each case.

						Dilute serum			Nitrogen as deci-normal acid in 50 c.c. of filtrate
						Heated	Unheated		
(a)	0.2 gm.	+	50 c.c.	+	20 c.c.	+	0 c.c.	=	19.3
(b)	0.2	+	50	+	15	+	5	=	17.6
(c)	0.2	+	50	+	10	+	10	=	15.2
(d)	0.2	+	50	+	5	+	15	=	15.0
(e)	0.2	+	50	+	0	+	20	=	13.3
(f)	0.2(heat-ed)	+	50	+	20	+	0	=	2.8

Tannic acid 50 c.c. added. Took 50 c.c. of filtrate.

Experiment 17.

Powder No. 6. Time $2\frac{1}{2}$ days. Substrate, casein 50 c.c. Serum Ox, dil. serum 1, normal saline 4. Total of 20 c.c. dil. serum in each.

						Dilute serum			Nitrogen as deci-normal acid in 50 c.c. of filtrate
						Heated	Unheated		
(a)	0.2 gm.	+	50 c.c.	+	20 c.c.	+	0 c.c.	=	19.0
(b)	0.2	+	50	+	10	+	10	=	15.5
(c)	0.2	+	50	+	0	+	20	=	13.0
(d)	0.2(heat-ed)	+	50	+	20	+	0	=	2.9

Tannic acid 50 c.c. added. 50 c.c. of filtrate taken for estimation.

Experiment 18.

Powder No. 8. Substrate, casein 50 c.c. Time $2\frac{1}{2}$ days. Serum Goat, dil. serum 1, normal saline 4. Total of 20 c.c. dil. serum in each.

						Dilute serum			Nitrogen as deci-normal acid in 50 c.c. of filtrate
						Heated	Unheated		
(a)	0.2 gm.	+	50 c.c.	+	20 c.c.	+	0 c.c.	=	21.0
(b)	0.2	+	50	+	10	+	10	=	17.4
(c)	0.2	+	50	+	0	+	20	=	14.6
(d)	0.2	+	50	+	20	+	0	=	2.8

Tannic acid 50 c.c. added. 100 c.c. of filtrate taken for estimation.

Comparative inhibitory effect of Sera from Different Animals.

With regard to the degree of inhibition manifested by normal sera Opie and Barker (1907) find it is not more marked with the serum of the species of animal from which the leucoprotease is taken than with other sera.

Pappenheim's results (quoted by Eisner 1909) do not agree altogether with Opie's. Pappenheim found the serum of the rabbit to be less active than that of the dog versus leucoprotease. He however agrees that birds' sera are little, if at all, active.

Delezenne using trypsin as ferment found considerable differences between various animal sera in their anti-power towards this ferment.

Mesnil (1903) working with actinodiastase as ferment found that sera of different species varied in their anti-power towards it and gives the following order of activity: sheep, goat, rabbit, birds.

As the evidence is at present then, normal sera from different animals show varying degrees of activity towards proteolytic ferments. This variability is thought by various observers to be a specific one but as regards leucoprotease at any rate, where two observers have worked on the same animals with the same ferment, they have not infrequently arrived at contradictory results, thus indicating that the differences between sera of individuals of the same species is as great as that between different species.

In my experiments I failed to find any very material difference between the activity of the sera of the various mammals on which I have experimented. In each case controls of maximum digestion, *i.e.* with all serum heated, and also two or more experiments with the fresh serum, one with a larger quantity than the other, were made. This enables one to see that one is not using such a dose of serum that it will give a maximum effect under the given conditions. The amount of serum added in each case is rendered equal by making up to constant amount with heated serum. The following experiments show some of my results:

Experiment 19.

Powder No. 8, 0.2 gm. Time 3½ days. Substrate, 50 c.c. casein sol. Serum, diluted serum 1, water 4. Total amount of dilute serum 20 c.c. in each case.

Dilute serum	Nitrogen as deci-normal acid in 25 c.c. of filtrate			
	Ox	Sheep	Goat	Rabbit
1. With 20 c.c. heated	8.0	7.9	7.65	7.4
2. With 10 c.c. fresh	6.8	6.3	6.15	6.4
3. With 20 c.c. fresh	—	5.25	5.3	5.6
4. Control	1.1	1.0	1.1	1.1

50 c.c. tannic acid sol. added. 25 c.c. of filtrate taken for estimation.

Experiment 20.

5 gm. of powder No. 10; ground with glass in sodium carbonate 0.25 % sol.; centrifuged; filtered and made up to 500 c.c.

Enzyme, solution as above 20 c.c. Time 2½ days. Substrate, casein solution 50 c.c. Dilute serum, serum 1, water 4. Total of 20 c.c. of dilute serum in each case.

Dilute serum	Nitrogen as deci-normal acid in 50 c.c. of filtrate			
	Ox	Sheep	Rabbit	Pigeon
All serum heated	7.4	7.5	7.3	—
10 c.c. dil. fresh serum	5.55	5.55	4.6	7.55
20 c.c. ,, ,,	4.6	4.4	3.4	—
Control	2.15	2.05	2.3	—

Tannic acid 50 c.c. Filtrate taken 50 c.c.

Experiment 21.

Powder No. 10, .02 gm. Time 2½ days. Substrate, casein sol. 50 c.c. Serum, 1 serum, 4 water. Total 20 c.c. in each case.

Dilute serum	Nitrogen as deci-normal acid in 50 c.c. of filtrate	
	Ox	Sheep
Serum all heated	16.15	16.9
10 c.c. fresh	15.35	14.65
20 c.c. ,,	13.15	11.8
Control	2.5	2.75

Tannic acid added 50 c.c. Took 50 c.c. of filtrate.

Experiment 22.

Enzyme. Powder No. 5, 0.1 gm. Substrate, gelatin 10 % 10 c.c. Time 3½ days. Diluent sodium carb. sol. 0.25 % 10 c.c. Serum (1—6 water), 10 c.c. in each case.

Dilute serum	Nitrogen as deci-normal acid in 50 c.c. of filtrate	
	Ox	Sheep
All serum heated	6.0	5.75
5 unheated	4.70	4.8
10 unheated	4.2	4.3
Control	1.8	1.7

50 c.c. tannic acid sol. added. 50 c.c. of filtrate taken.

Immunity.

Achalme (1901) increased the anti-tryptic power of serum in guinea-pigs by injecting them intra-peritoneally with trypsin. Dean (1901) immunised goats and geese with trypsin but obtained only a trifling increase in anti-tryptic power.

Levene and Stookey (1903) found that the serum of trypsin-immunised rabbits was *markedly* more powerful as anti-body to tryptic

digestion. The animals were treated for eight weeks with a dose increasing to 2 c.c. intravenously.

Landsteiner (1907) failed to increase the anti-tryptic power of serum notwithstanding a lengthy immunisation.

Jochmann and Kantorowicz (1908) immunised rabbits with both trypsin and leucoprotease and found an increase of thirtyfold in the power of the serum. The sera of the animals injected with either trypsin or leucoprotease contained an anti-body for both enzymes and saturation of the sera with the one enzyme exhausted it of its neutralising power for both.

Bergmann and Bamberg (1908) doubled the anti-tryptic power of dog's serum by two months' immunisation.

Döblin (1909) failed to increase the anti-power by injecting trypsin into rabbits whereas Meyer (1909) obtained in the same animals an increased anti-tryptic power after the injection of trypsin but not after trypsinogen or kinase.

My own results with leucoprotease are parallel with those of Dean, there being according to the figures (Ex. 23 and 24 below) a slight increase of anti-power. As however the nutrition of the goats was somewhat influenced by the course of injections, *i.e.* loss of weight was noticed, I am inclined to think that such slight variation as is shown in the course of my experiments is as likely if not more likely to be caused by such influences than by any process of true immunisation. Had I used rabbits or dogs the results might have been different.

The goats were treated by subcutaneous injection of preparations of the pus-powder, prepared as mentioned above. This was either given in the form of suspensions in normal saline or sodium carbonate 0.25 % sol. or by extracts of the powder made by grinding it with glass in saline or sod. carb. sol., centrifuging and filtering. This treatment the animals bore well. The early injections were followed by a good deal of reaction causing firm nodules to appear which were however subsequently absorbed. After the later injections although the dose was considerably increased, this was far less marked. The doses injected are shown in the table as approximate amounts of powder used. I have given the actual ammonia figures of the estimation as well as the percentages of inhibition of the serum.

In estimating this anti-tryptic action the following method was used. 0.2 gm. of the powder was allowed to act in the presence of 2 c.c. and 4 c.c. of the pure serum on 50 c.c. of 2.5 casein solution in the sodium carbonate solution 0.25 %. In each case the serum was

*Experiment 23.**Protocols of estimations of anti-action of serum of immunised goats.*

	Goat 1 B	Goat 2 B	Goat 1 L
	Dates :—8. 3. '09	9. 3. '09	26. 2. '09
A.	21·0	19·9	19·3
B.	17·4 (20)	16·3 (21)	15·2 (24)
C.	14·6 (35)	13·7 (36)	13·3 (36)
D.	2·8	2·8	2·8
	Dates :—26. 3. '09	26. 3. '09	13. 3. '09
A.	18·8	18·3	19·4
B.	14·55 (26)	15·05 (20)	15·6 (22)
C.	12·0 (40)	13·1 (32)	12·6 (40)
D.	2·2	2·1	—
	Dates :—14. 4. '09	14. 4. '09	26. 3. '09
A.	16·3	15·7	18·8
B.	14·25 (14)	13·5 (16)	—
C.	11·5 (33)	11·4 (31)	12·45 (38)
D.	1·8	1·9	2·2
	Dates :—24. 4. '09	24. 4. '09	—
A.	17·05	—	—
B.	14·5 (17)	14·25 (19)	—
C.	11·9 (34)	12·5 (30)	—
D.	2·3	2·15	—
	Dates :—17. 5. '09	17. 5. '09	—
A.	17·65	17·45	—
B.	14·0 (25)	14·15 (23)	—
C.	12·0 (39)	11·75 (40)	—
D.	3·25	3·1	—
	Dates :—4. 6. '09	4. 6. '09	—
A.	14·85	15·55	—
B.	11·8 (25)	11·85 (28)	—
C.	9·4 (43)	—	—
D.	2·4	2·4	—
	Dates :—15. 6. '09	15. 6. '09	—
A.	18·9	19·6	—
B.	14·4 (28)	14·45 (30)	—
C.	11·4 (46)	11·4 (48)	—
D.	2·65	(2·65)	—

First figures = nitrogen as c.c. N/10 acid in 50 c.c. of filtrate. Figures in brackets =
 % reductions. 50 c.c. tannic acid sol. added.

Experiment 24.

Goat 1 L.

Days	Amount of powder injected	Percentage reductions	
		With 2 c.c.	With 4 c.c.
1	—	24	36
11	0.05 a	—	—
12	0.2 a	—	—
13	0.3 b	—	—
16	0.4 b	22	40
29	—	—	38

Goat 1 B.

1	—	20	35
13	0.2 b	—	—
18	0.2 b	—	—
19	—	26	41
26	0.2 c	—	—
31	0.2 c	—	—
37	0.4 b	14	33
39	0.8 b	—	—
48	—	17	34
71	0.6 d	25	39
80	1.2 d	—	—
89	1.6 e	25	43
100	0.08 IV	28	46

Goat 2 B.

1	—	21	36
12	0.2 b	—	—
17	0.2 b	—	—
18	—	20	32
25	0.2 c	—	—
30	0.2 c	—	—
36	0.4 b	16	31
38	1.0 b	—	—
47	—	19	30
70	0.6 d	23	40
80	1.2 d	—	—
89	1.6 e	28	—
99	0.1 IV	30	48

a—1 % extract in normal saline.

b—suspension in sodium carbonate solution 0.25 %.

c—suspension 1 % in normal saline.

d—1 % extract in sodium carbonate solution 0.25 %.

e—4 % suspension in normal saline.

IV—1 % extract in sodium carbonate solution 0.25 % given intravenously.

diluted in the proportion of 1 to 4 of normal saline and the total amount of this dilute serum was in each case made up to 20 c.c. by similarly diluted serum previously heated to 90° C. In the preceding table the numbers given in the horizontal columns A, B, C, D represent the following:

- A. Maximum digestion with all serum, 20 of dilute, heated.
- B. With 2 c.c. serum, 10 c.c. of the dilute serum, fresh.
- C. With 4 c.c. of the serum, 20 c.c. of dilute, fresh.
- D. Control, enzyme powder heated, serum all heated, 20 c.c. of dilute.

The figures in brackets after B and C in Expt. 23 represent percentage reduction of activity.

Before treatment all three goats gave percentage reduction figures between 20 % and 24 % for 2 c.c. of fresh serum and between 35 % and 36 % for 4 c.c. of fresh serum, while at the end of the course of injections the percentage reduction figures for goats 1 B and 2 B were between 28 % and 30 % for 2 c.c. of fresh serum and between 46 % and 48 % for 4 c.c. of fresh serum.

During the course of estimation it will be noticed that on one occasion the percentage reduction figures fell to between 14 % and 16 % for 2 c.c. of fresh serum and 33 % and 31 % for 4 c.c. of fresh serum. I think it is likely that the 14 % and 16 % are for some reason under-estimations but otherwise the 2 c.c. and 4 c.c. estimations run pretty fairly parallel.

In conclusion I wish to express my thanks to Dr Martin, Director of the Institute, and Professor Leathes, Head of the Laboratories for Pathological Chemistry. To Professor Leathes I am indebted for much valuable advice and assistance throughout the progress of the work.

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GUINEA-PIGS AS CHRONIC CARRIERS OF AN ORGANISM BELONGING TO THE FOOD-POISONING GROUP.

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(From the Lister Institute of Preventive Medicine.)

A GOOD deal of research has been devoted during the last few years to the subject of "carriers" in connection with various diseases of man, but up to the present time less attention has been directed to the existence of the same condition in animals.

In November last an epizootic broke out amongst a stock of about 500 guinea-pigs at the Lister Institute and all but 21 of them died. A bacillus of the food-poisoning group was closely associated with the epizootic¹; its relationship to the transmission of the disease is discussed in another paper, but from its frequent occurrence in animals during the epizootic, in the intestines as well as in the organs, an examination of the faeces of the survivors of the epizootic appeared to be desirable.

Two of the animals were killed for other purposes, while of the remaining nineteen, ten had been tested for evidence of acquired immunity by the subcutaneous inoculation of a broth culture of the bacillus above mentioned. The subcutaneous inoculation of the bacillus is often followed by its appearance in the intestine so that any carriers found amongst these ten animals could not be called natural carriers, and the results of the examination of their faeces are therefore not included in Table I, which shows the results of the examination, at different dates between January and May, of the faeces of nine of the guinea-pigs which survived the epizootic.

The pellets of faeces were incubated in dulcete broth at 37° C. for 48 hours, and from the tubes that showed gas a loop of broth was spread on MacConkey lactose plates to which saccharose had been added².

¹ The identification of the bacillus will be discussed later.

² The use of plates containing both lactose and saccharose was found to facilitate the isolation of the bacillus, since many organisms present in faeces which do not ferment lactose but produce acid in saccharose are excluded by this method.

TABLE I.

Results of examination of faeces of nine of the survivors of the epizootic.

Date of examination	Results of examination : occurrence of bacillus in the faeces			
1. 1. 1910 to 8. 4. 1910	Guinea-pig "A"—8 examinations, all positive.			
	Guinea-pig "B"	Guinea-pig "C"	Guinea-pig "D"	Guinea-pig "E"
5. 4. 1910	—	—
14. „	+	—
19. „	+	+
22. „	—
23. „	+	—
24. „	—	—
25. „	+	—
27. „	—	—
28. „	—	+
2. 5. 1910	+
3. „	+	—	+	+
4. „	—	—	—	—
9. „	+	—	—	—
30. „	+	...	—	—

The four other survivors examined on five occasions with "D" and "E" gave negative results throughout.

The dulcete bile salt broth strongly favours the growth of bacilli of the food-poisoning group in a mixture of these organisms with lactose fermenters, but it was rather interesting to find that specimens from the carriers "A" and "B" frequently gave a pure culture of the bacillus on the plates; plates similarly inoculated from carriers "C," "D" and "E" generally showed many lactose fermenters and very few non-fermenters. In two of the early examinations of faeces from "A" the pellets were emulsified in salt solution and a loop of emulsion at once spread on a lactose-saccharose MacConkey plate with the result that an almost pure culture of the bacillus was obtained. In human typhoid carriers an almost pure culture of the typhoid bacillus has been frequently reported and the observation above suggests that the normal balance of intestinal flora is here similarly overthrown, resulting in the diminution or disappearance of lactose fermenting organisms.

From this table we see that in the case of animals "D" and "E" the bacillus was recovered once in five times (*i.e.* 20%), "C" gave the same percentage over ten examinations, while "B" gave eight positive results in thirteen examinations (= 61%) and "A" was examined eight times and gave positive results on every occasion (= 100%).

Thus out of nine survivors of the epizootic whose faeces were tested five proved to be carriers of the bacillus.

agglutination of the bacillus by the serum of the carrier guinea-pigs.

It next became of interest to determine whether the serum of these animals agglutinated the bacillus, and it was found that the serum of all four examined agglutinated the bacillus in a dilution of 1 in 50 or 1 in 100, whereas of six stock guinea-pigs tested, one only gave agglutination in a dilution of 1 in 20. The details appear in Table II.

TABLE II.

Agglutination limits with serum of "carriers" and of normal animals tested against the bacillus.

Guinea-pig tested	Result of agglutination test with serum from each animal in various dilutions			
	Dilution: 1 in 20	50	100	500
B	+	+	+	-
C	+	+	-	-
D	+	+	-	-
E	+	+	+	-
stock animal 1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	+	-	-	-

Immunity of survivors of the epizootic.

Interesting results were obtained from the inoculation of varying doses of broth cultures of the bacillus into ten of the survivors from the epizootic. A strain of the bacillus found in the organs of a guinea-pig dying during the epizootic was grown in broth and after 20 hours varying doses were injected subcutaneously into each of the ten animals. The lethal dose for stock animals of the particular culture used was 0.00001 c.c. of a 24 hours' broth culture. Of the ten animals only three died; the bacillus was not recovered post-mortem in one case (No. 6, see Table III) and it is probable that it died from some other cause. From animal No. 8 the bacillus was recovered at autopsy. These results justify the conclusion that eight of the ten animals showed a definite degree of immunity to varying multiples of the minimal lethal dose of the bacillus.

The immunity of the carriers to subcutaneous inoculation of the bacillus has not yet been tested but this will be done after further observations have been made on the duration of the excretion of the bacillus.

TABLE III.

Experiments to ascertain the degree of acquired immunity of survivors from the epizootic against a strain of the bacillus of which .00001 c.c. of a 24 hours' broth culture was fatal to a stock animal when injected subcutaneously.

Guinea-pig	Dose of 24 hours' broth culture injected subcutaneously	Result
1	1 c.c.	Died in 18 days. (Control stock animals given this dose died in 14 hours.)
2	1	Survived.
3	.5	"
4	.1	"
5	.01	"
6	.001	Died. The bacillus was not recovered postmortem.
7	.001	Survived.
8	.001	Died. The bacillus was recovered.
9	.00001	Survived.
10	.00001	"

Pathogenicity of the bacillus excreted by the carriers.

A number of the strains of the bacillus recovered from the organs of animals dying during the epizootic were fatal when given subcutaneously to normal guinea-pigs in very small doses. The pathogenicity of the strain of the bacillus excreted in the faeces of one of the carriers was tested and found to be on the whole somewhat less than that of the original epizootic strain. A dose of .001 of a 24 hours' broth culture of the original bacillus recovered during the epizootic was invariably fatal to stock animals, death ensuing generally after five days, whereas of the three stock animals tested with this dose of the carrier's bacillus, one lived and the other two died after periods of 13 and 20 days.

TABLE IV.

Experiments to determine the pathogenicity of the bacillus excreted by one of the carriers, when inoculated subcutaneously into guinea-pigs.

Dose of 24 hours' broth culture	Result	Result of cultures + = the bacillus recovered
.5 c.c.	Died, 5 days	Heart-blood +
.001	Died, 20 days	"
.001	Died, 13 days	"
.001	Survived	—
.0001	"	—

A dose of .001 c.c. of a similar culture of the original bacillus recovered from the epizootic killed in 5 days.

Identification of the bacillus associated with the epizootic and occurring in the carriers' faeces.

The cultural reactions, fermentation of carbohydrates, etc., were those of the food-poisoning group; the bacillus was not agglutinated by the serum of a rabbit immunised against the *B. enteritidis* (Gaertner) and therefore evidently belonged to the paratyphoid B. group. It was then tested against the sera of two rabbits immunised against the *B. aertryck* and the *B. paratyphosus* B. respectively (see Table V).

TABLE V.

Identification of the bacillus by agglutination and absorption tests.

"A" is the bacillus recovered from the faeces of carrier "B."

Agglutination results with various dilutions of the serum of a rabbit immunised against *B. aertryck*.

	Dilution of serum: 1 in 40	2000	5000	10,000	Control
<i>B. paratyphosus</i> B.	+	+	+	—	—
<i>B. aertryck</i>	+	+	+	—	—
<i>B. "A"</i>	+	+	+	—	—

Agglutination results of similar experiment with the same serum after absorption with the *B. paratyphosus* B.

	Dilution of serum: 1 in 40	2000	5000	Control
<i>B. paratyphosus</i> B.	—	—	—	—
<i>B. aertryck</i>	+	+	—	—
<i>B. "A"</i>	+	+	—	—

Agglutination results with various dilutions of the serum of a rabbit immunised against the *B. paratyphosus* B.

	Dilution of serum: 1 in 40	500	1000	2000	5000	Control
<i>B. paratyphosus</i> B.	+	+	+	+	—	—
<i>B. aertryck</i>	+	+	+	+	—	—
<i>B. "A"</i>	+	+	+	+	—	—

Agglutination results of similar experiment with the same serum after absorption with the *B. aertryck*.

	Dilution of serum: 1 in 40	500	1000	2000	Control
<i>B. paratyphosus</i> B.	+	+	—	—	—
<i>B. aertryck</i>	—	—	—
<i>B. "A"</i>	—	—	—

The agglutination results do not permit the bacillus to be definitely assigned to either the *aertryck* or the paratyphoid B. group, for the serum of the rabbit immunised against the *B. aertryck* agglutinated that bacillus and the *B. paratyphosus* B. equally, and conversely the serum of the rabbit immunised against the *B. paratyphosus* B. agglutinated that bacillus and the *B. aertryck* equally.

Accordingly from these sera the heterologous agglutinins were in each case absorbed with an emulsion of the corresponding heterologous

bacillus to such a degree that each serum in a dilution of 1 in 40 no longer agglutinated the heterologous bacillus whilst retaining enough of the homologous agglutinins to agglutinate the homologous bacillus in a dilution of 1 in 500 or over. A clear difference between the *B. paratyphosus* B. and the *B. aertryck* then appeared in their agglutination reactions to these "absorbed" sera, as had already been demonstrated by Bainbridge (*Journ. of Pathology and Bacteriology*, Vol. XIII., 1909, p. 443).

The bacillus recovered during the epizootic and the bacillus recovered from the carriers' faeces gave the same results as the *B. aertryck*, so that the bacillus is indistinguishable by any test at present available from the *B. aertryck* or the *B. suipestifer*¹.

Contact experiments.

The carriers "B" and "D" were placed in separate cages and into each cage were put two normal stock guinea-pigs. One of the contacts placed with "B" died but the bacillus was not recovered from the organs or the intestinal contents post-mortem. The other three contact animals lived and apparently remained healthy for the period of two months they were under observation; an examination of the faeces was made on two occasions but the bacillus was not recovered.

SUMMARY.

A stock of 500 guinea-pigs at the Lister Institute was attacked by an epizootic and only 21 survived. These survivors showed definite immunity to a bacillus of the food-poisoning group (indistinguishable from *B. aertryck* and the *B. suipestifer*) recovered frequently from animals dying during the epizootic. Five of them have been proved to be carriers excreting the bacillus intermittently five months later and the serum of all of them agglutinates the bacillus. Spread of infection apparently did not occur amongst contacts placed with these carriers in the few experiments carried out.

I have to thank the staff of the Lister Institute for much assistance during this research and Dr F. A. Bainbridge for much practical help in the determination of the bacillus by the methods described in his paper on members of this group.

¹ Bainbridge (*vide supra*) has also shown that the *B. suipestifer* and the *B. aertryck* are indistinguishable by agglutination and absorption tests.

THE PROCESS OF DISINFECTION BY CHEMICAL
AGENCIES AND HOT WATER.

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(With 21 Text Figures.)

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I. INTRODUCTION.

Krönig and Paul (1897) were the first to study the process of disinfection by making quantitative observations at intervals during its progress. Their figures showed that disinfection proceeded in an orderly manner and that the rate diminished as the number of survivors became less. Ikéda (1897) attempted to express their results by an empirical formula.

In 1907—1908, Madsen and Nyman, and H. Chick working simultaneously but independently at this subject, found that during the process of the disinfection of anthrax spores by chemical agents (mercuric chloride and phenol respectively) the concentration of spores remaining alive varied logarithmically with the time, and that therefore the number of anthrax spores destroyed per unit time was proportional to the number present in a unit volume of the medium at that moment. An analysis of the figures published by Krönig and Paul showed the same relationship. Tables Ia (H. C. 1908, p. 99) and Ib (K. and P. 1897, p. 26) are examples of the progress of disinfection of anthrax spores with phenol and mercuric chloride respectively; the corresponding curves in Figs. 1a and 1b, where logarithms of concentration of survivors are plotted against time, show the logarithmic relation graphically.

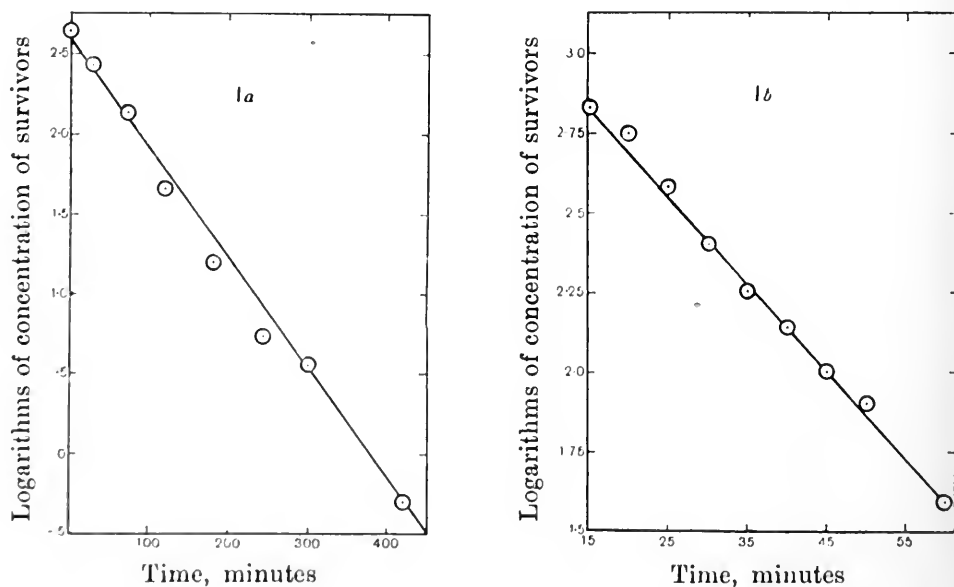


Fig. 1. (a) Disinfection of anthrax spores with 5% phenol at 33.3°C. (H. C. 1908, Table II, p. 99).

(b) Disinfection of anthrax spores with 0.11% mercuric chloride at 18°C. (Krönig and Paul, 1897, Table IX, p. 26).

TABLE I a.

Disinfection of Anthrax Spores with 5 % phenol at 33.3° C.

(See H. C. (1908), Table II, p. 99.)

Time, minutes = t	Mean number of bacteria surviving in one drop of dis- infection mixture, = N	$\text{Log}_{10} N$	* $K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0 = t_0	439 = N_0	2.642	—
30	275.5	2.439	.0068
75	137.5	2.138	.0067
120	46	1.663	.0082
180	15.8	1.199	.0080
246	5.45	.736	.0077
300	3.6	.556	.0069
420	0.5	— .301	.0070

* In this and all subsequent tables, values of K from the expression $\frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$ are calculated with Briggs' logarithms in place of natural logarithms.

TABLE I b.

Disinfection of Anthrax Spores with 0.11 % mercuric chloride at 18° C.

(See Krönig and Paul (1897), Table IX, p. 26.)

Time, minutes = t	Mean number of bacteria surviving = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
10 = t_0	2027 = N_0	3.307	—
15	672	2.827	.096
20	564	2.751	.056
25	382	2.582	.048
30	251	2.400	.045
35	179	2.253	.042
40	138	2.140	.039
45	101	2.004	.037
50	80	1.903	.035
60	39	1.591	.034
70	6	.778	.042
80	3	.477	.040

The conclusion arrived at by Madsen and Nyman and also by myself was that if the disinfectant be present in large excess relative to the bacteria, disinfection proceeds in the same manner as a reaction of the first order, that is to say, as a unimolecular reaction, or, what amounts to the same thing, as a bimolecular reaction with one reagent in excess.

The reason for it so doing must, apparently, be sought for in the same direction as in the case of the hydrolysis of sugar by water or the decomposition of hydrogen arsenide by heat, which are both reactions of the first order. The explanation offered by the physical chemist is that there is no permanent dissimilarity between the molecules, but that at any one time only a proportion of them is in the condition to undergo the dissociation or chemical union. This temporary increased susceptibility is believed to be occasioned by differences in the internal energy of the molecules. This interpretation has the advantage of affording a possible explanation of the influence of temperature upon the rate of reaction.

It would appear that with anthrax spores we are dealing with a population possessing but slight permanent differences in regard to their behaviour towards disinfectants, and that the fact that the whole number is not killed off in the same time is not, as has been supposed, due to permanent variations in resistance of individuals but, as in the chemical analogies given above, occasioned by temporary energy changes in the molecules of which the bacterial proteins are composed.

In the case of *B. paratyphosus*, the vegetative form worked with (H. C. *loc. cit.*), broth cultures, obtained by successive sub-culturing at frequent intervals, behaved as anthrax spores on disinfection with phenol and the nature of the disinfection process was similarly explained. The observations made upon 24 hours' cultures of *B. paratyphosus*, however, indicated the existence in this case of some disturbing factor which caused the process of disinfection to depart from the simple law found to apply in the case of spores and "young" cultures.

These interpretations, put forward in the previous paper already referred to, have been criticised by Eijkman (1909), and by Hewlett (1909) in his Milroy Lectures. Although these criticisms can, I think, be met by appeal to the facts already published, they have stimulated me to perform some further experiments. These experiments will be presented first and the points raised by my critics will be dealt with subsequently.

II. DISINFECTION WITH PHENOL.

(1) *Method employed.*

The method employed for studying the reaction velocity of disinfection in this instance was the same as that used in the previous paper (H. C. 1908). A measured quantity (five drops from a standard

capillary pipette = .08 c.c.) from a 24 hours' broth culture of the organism selected was added to a tube containing 5 c.c. phenol solution immersed in a water bath maintained at 20° C. This disinfection tube was fitted with a capillary pipette by means of which samples could be withdrawn and a definite number of drops taken for agar or gelatine plate cultures at successive intervals of time. Three plates were poured at each time of sampling, necessitating the help of two assistants. The sample drops were dropped into 0.5 c.c. of sterile distilled water, previously placed upon the plates; this lessened the error arising from the impossibility of pouring all three plates simultaneously. The plates were counted after 48 hours' incubation at 37° C. in the case of agar, or after four or five days' at 20° C. in the case of gelatine plates, and the progress of the disinfection reaction was studied by thus enumerating the surviving bacteria at successive periods of the process.

(a) *Experiments with B. typhosus.*

Tables II and III give the results of two experiments with *B. typhosus* and 0.6 % phenol at 20° C. Gelatine¹ plates were used in

TABLE II.

Disinfection of B. typhosus with 0.6 % phenol at 20° C.

EXP. 8. 1. '10.

Time, minutes = <i>t</i>	Amount of sample taken, drops	Numbers on plates (gelatine)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = <i>N</i>	Log ₁₀ <i>N</i>	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0.5 = <i>t</i> ₀	1	236 218 240	231.3	231.3 = <i>N</i> ₀	2.364	—
1.5	1	188 215 167	190	190	2.279	.085
2.6	1	160 157 184	167	167	2.223	.067
4.6	2	215 242	228.5	114.2	2.058	.075
6.6	2	139 172 156	155.7	77.8	1.891	.077
10.5	3	100 93 91	94.7	31.6	1.500	.086
15	3	33 26 43	34	11.3	1.053	.090
20	5	14 14 8	12	2.4	.380	.102
30	10	4 3	3.5	.35	-.456	.096
Mean						.086

¹ Gelatine plates were substituted for agar in all the later experiments in spite of the obvious disadvantages. There is some risk of error in the use of agar owing to the necessarily high temperature at which the plates are poured, and comparative experiments showed that higher numbers were invariably obtained by using gelatine, although the proportion remained fairly constant.

TABLE III.

Disinfection of B. typhosus with 0.6 % phenol at 20° C.

Exp. 14. 1. '10.

Time, minutes = t	Amount of sample taken, drops	Numbers on plates (gelatine)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0.5 = t_0	1	779 717 645	714	714 = N_0	2.854	—
1.5	1	584 571	577.5	577.5	2.762	.092
2.5	1	406 526 451	461	461	2.664	.095
4.5	1	313 215 288	272	272	2.435	.105
6.5	1	136 149 145	143.3	143.3	2.156	.116
10	2	47 162 155	121.3	60.6	1.782	.113
20	5 (10)	35 23 54	— —	4.48	.651	.113
Mean						.106

both experiments. Disinfection, as in the case of anthrax spores, proceeded so that the number of survivors in unit volume varied logarithmically with the time; the logarithms of these numbers, when plotted against time, also gave straight lines (Fig. 2). The velocity constant, $K \left(= \frac{I}{t_n - t_0} \log \frac{N_0}{N_n} \right)$, calculated from the experimental numbers, is given in the last column of either table, and shows fair constancy in value.

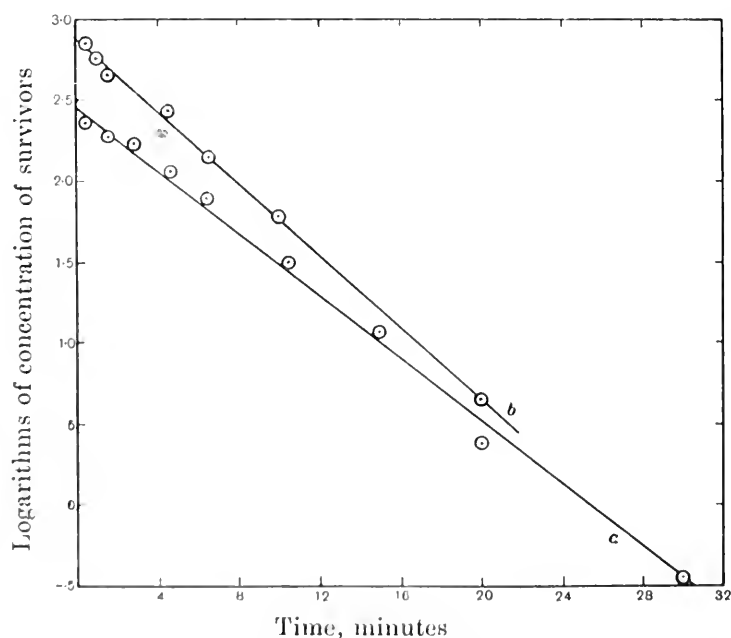


Fig. 2. Disinfection of *B. typhosus* with 0.6 % phenol at 20° C.
 (a) Exp. 8. 1. '10, Table II. (b) Exp. 14. 1. '10, Table III.

(b) Experiments with *B. coli commune*.

Tables IV and V give the results of two experiments with *B. coli commune* and 0·5 % phenol at 20° C., in the one case agar and in the other gelatine plates being used. 24 hours' cultures in broth at 37° C.,

TABLE IV.

Disinfection of B. coli commune with 0·5 % phenol at 20° C.

EXP. 15. 2. '10.

Time, minutes = <i>t</i>	Amount of sample taken, drops	Numbers on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = <i>N</i>	Log ₁₀ <i>N</i>	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0·8	1	593 623	608	608	2·784)	—
1·9 = <i>t</i> ₀	1	286 354 256	299	299 = <i>N</i> ₀	2·476	—
2·9	1	229 219 301	249·7	249·7	2·397	·079
4·25	1	193 209 217	206·3	206·3	2·314	·069
5·75	1	192 172 158	174	174	2·240	·061
7·5	{ 1	127	— }	129·3	2·112	·065
	{ 2	261	— }			
10	2	144 207	175·5	87·75	1·943	·066
15	{ 3	121	— }	47·7	1·678	·061
	{ 5	261	— }			
20	{ 5	116	— }	20·73	1·317	·064
	{ 10	195	— }			
30	10	51 20	35·5	3·55	·550	·068
40	20	7 13	10	·5	—·301	·073
Mean						·067

TABLE V.

Disinfection of B. coli commune with 0·5 % phenol at 20° C.

EXP. 21. 3. '10.

Time, minutes = <i>t</i>	Amount of sample taken, drops	Numbers on plates (gelatine)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = <i>N</i>	Log ₁₀ <i>N</i>	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0·6	1	645 623 648	639	639	2·805)	—
1·5	1	565 564 639	589	589	2·770)	
2·5 = <i>t</i> ₀	1	436 470 475	460	460 = <i>N</i> ₀	2·663	—
3·75	1	425 395 364	395	395	2·597	·053
5	1	340 329 351	340	340	2·531	·053
7	2	493 513 498	501	250	2·398	·059
10	{ 2	387 416	— }	196	2·292	·049
	{ 10	2080 1830	— }			
15	2	204 273	238	119	2·075	·047
20	20	1670 1780	1725	86·2	1·935	·042
Mean						·051

made from a stock agar culture grown at room temperature, again served as the material to be disinfected and the method was exactly as above described.

The results are similar to those obtained in the case of *B. typhosus*; as disinfection proceeds a logarithmic relation is approximately maintained between the time of disinfection and the concentration of surviving bacteria.

In some cases for a very short period (usually less than two minutes) at the beginning of the experiment there was some irregularity. Numbers counted during this period are not plotted in Fig. 3 or used for calculating the velocity constants in Tables IV and V. I am inclined to attribute this to a difficulty with this species in obtaining perfect mixing and accurate sampling during the first moments of an experiment. The variation was not consistent in one sense, in some cases the number of bacteria counted was larger, in others smaller than was to be expected. Eijkman's (1909) results which differ from the above will be referred to later (see p. 249).

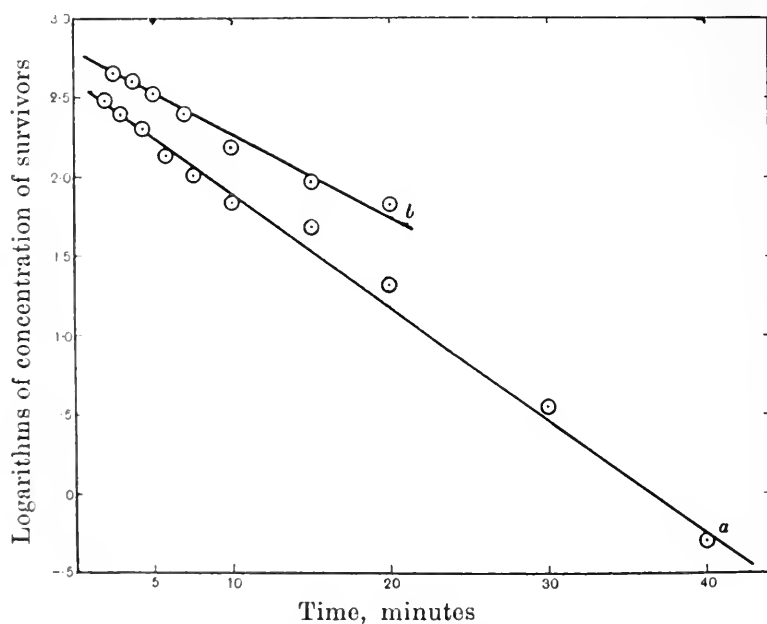


Fig. 3. Disinfection of *B. coli commune* with 0.5 % phenol at 20° C.
(a) Exp. 15. 2. '10, Table IV. (b) Exp. 21. 3. '10, Table V.

(c) *Staphylococcus pyogenes aureus*.

Disinfection proceeds logarithmically except for a period at the beginning, during which disinfection is extremely slow. This period of lag lasted in all cases for four minutes, see Figs. 4 and 5, and was

consistently present in contradistinction to the irregularity frequently noticed in the case of *B. coli commune*. At the end of this period disinfection proceeded in approximate accordance with the logarithmic law.

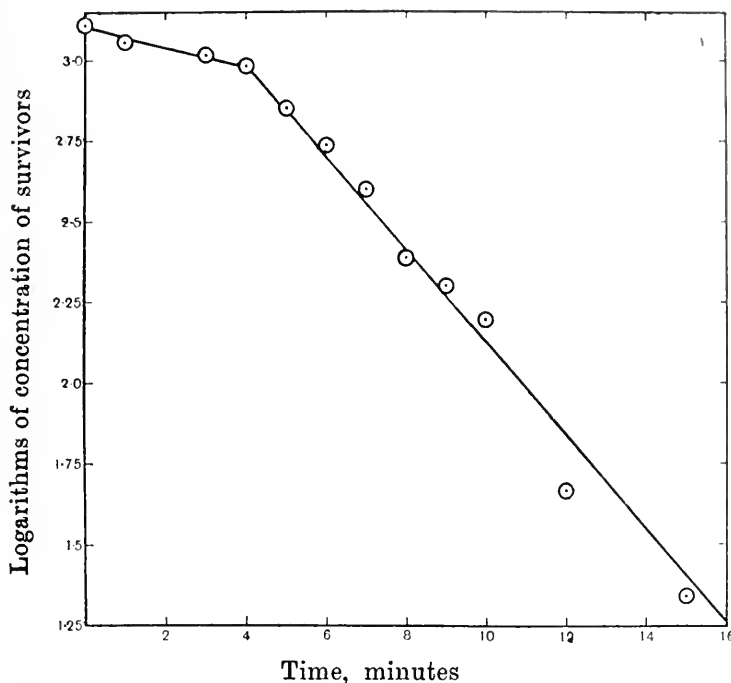


Fig. 4. Disinfection of *Staphylococcus pyogenes aureus* with 0·6 % phenol at 20° C.
(Exp. 6. 4. '09, Table VI.)

TABLE VI.

Disinfection of Staphylococcus pyogenes aureus with 0.6 % phenol at 20° C.

Exp. 6. 4. '09.

Time, minutes = t	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
Control	1	1369 1218	1293	1293	3.112	—
1	1	1160 1120 1143	1141	1141	3.057	—
3	1	974 1064 1093	1044	1044	3.019	—
4= t_0	1	986 929 940	952	952= N_0	2.979	—
5	1	693 669 762	708	708	2.850	.129
6	2	1062 1048 1148	1086	543	2.735	.122
7	3	1253 1167 1187	1202	401	2.603	.125
8	3	728 731	729.5	243.2	2.386	.148
9	5	1113 912	1012.5	202.5	2.306	.135
10	5	761 691 890	780.7	156.1	2.193	.131
12	10	626 326 410	454	45.4	1.657	.165
15	10	220 216	218	21.8	1.338	.149
					Mean	.136

TABLE VII.

Disinfection of *Staphylococcus pyogenes aureus* with 0·6 % phenol at 20° C.

Exp. 16. 3. '09.

Time, minutes = <i>t</i>	Amount of sample taken, drops	Numbers counted on plates (agar)			Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = <i>N</i>	Log ₁₀ <i>N</i>	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
Control	1	1222	1140		1180	1180	3·072	—
1	1	1005	978	1011	998	998	2·999	—
2	1	958	995	944	966	966	2·985	—
3	1	794	878	889	854	854	2·931	—
4 = <i>t</i> ₀	2	1498	1400	1570	1489	744 = <i>N</i> ₀	2·872	—
5·1	3	2039	1882	1649	1857	619	2·792	·073
7	8	2723	2586	2100	2469	309	2·490	·127
10	10	961	1172	1550	1228	122·8	2·089	·130
15	{ 10	315	247		—	29·1	1·464	·128
	{ 6	187			—			
Mean								·114

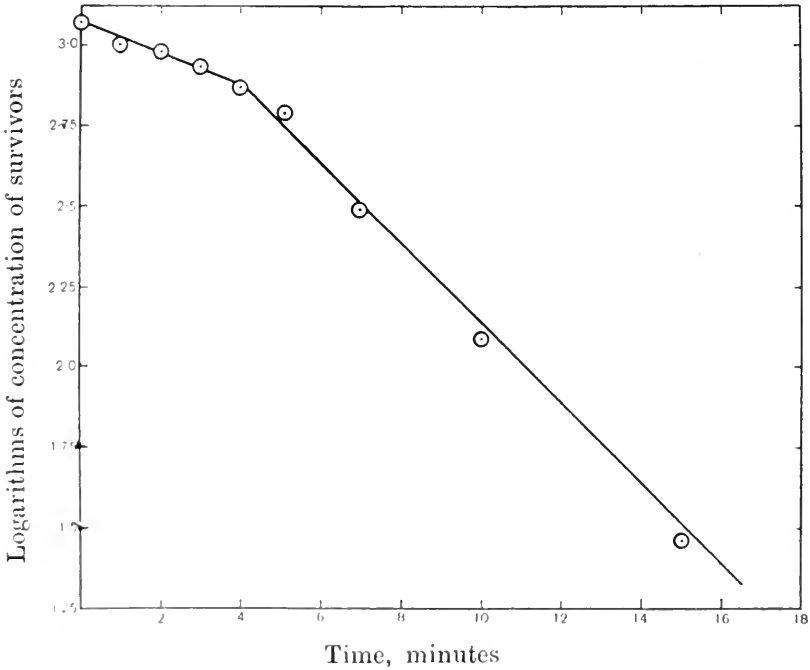


Fig. 5. Disinfection of *Staphylococcus pyogenes aureus* with 0·6 % phenol at 20° C. (Exp. 16. 3. '09, Table VII.)

The results of two experiments with *Staphylococcus py. aur.* and 0·6 % phenol at 20° C. are given in Tables VI and VII. For reckoning the value of the velocity constant in these tables the initial concentration of bacteria is taken as that obtaining at the end of the “period of

lag," as determined from the curves in Figs. 4 and 5 respectively, where logarithms of concentration of survivors are plotted against time. The results of two additional experiments are given in Table VIII.

TABLE VIII.

Disinfection of Staphylococcus pyogenes aureus with 0.6 % phenol at 20° C.

EXP. I, 30. 3. '09.

Time, minutes = <i>t</i>	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = <i>N</i>	Log ₁₀ <i>N</i>	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
3	1	310	310	310	2.491	—
4 = <i>t</i> ₀	1	284 224 236	248	248 = <i>N</i> ₀	2.394	—
5	1	120 128 127	125	125	2.097	.30
6	1	107 109	108	108	2.033	.18
7	2	95 88	91.5	45.7	1.660	.24
8	2	63 47	55	27.5	1.439	.24
9	2	36 36	36	18	1.255	.23
10	3	13 14 33	20	6.66	0.823	.26
12	5	26 25	25.5	5.1	.708	.21
15	5	17 19 18	18	3.6	.556	.17
Mean						.23

EXP. II, 13. 3. '09.

1	1	663 641 633	646	646	2.810	—
3 = <i>t</i> ₀	1	246 260 254	253	253 = <i>N</i> ₀	2.403	—
5	1	42 49 41	44	44	1.643	.38
7	2	40 24 20	28	14	1.146	.31
10	2	4 6 7	5.66	2.83	.452	.28
Mean						.32

In Table IX are given the results of two experiments with *Staphylococcus py. aur.* and 0.6 % phenol, in which comparison is made between the disinfection of bacteria grown at 37° C. (Exp. *a*) and bacteria grown at 42° C. (Exp. *b*), the conditions of experiment being otherwise identical. Disinfection in the first case was consistent with the experiments described above, but the bacteria grown at the higher temperature displayed as a whole a greatly increased resistance, and disinfection was so slow that it was impossible to study it within the time of the experiment. A similar phenomenon was noticed in the case of disinfection of *B. typhosus* with phenol by Rideal and Walker (1903, p. 431), and by Martin and the author (1908, p. 661).

TABLE IX.

Disinfection of *Staphylococcus pyogenes aureus* with 0·6 % phenol at 20° C.

Experiments to show difference in resistance of a culture grown at 37° C.
and one grown at 42° C.

Exp. 23. 3. '09. (a) Culture grown at 37° C.						
Time, minutes = <i>t</i>	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = <i>N</i>	Log ₁₀ <i>N</i>	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
Control	1	656 679	667	667	2·824	—
1	1	600 709 554	621	621	2·793	—
2	1	659 616 516	597	597	2·776	—
3	1	587 616 613	605	605	2·782	—
4 = <i>t</i> ₀	1	510 544 460	505	505 = <i>N</i> ₀	2·703	—
5	2	765 932	848	424	2·627	·076
7	3	1115 847 1017	993	331	2·520	·091
10	{ 10	1488 1410	—	150·5	2·177	·088
	{ 8	1317	—			
Mean						·085

(b) Culture grown at 42° C.						
Control	1	116	116	116	2·064	—
2	1	80 102 101	94·3	94·3	1·975	—
3	1	117 91	104	104	2·017	—
5	2	171 190 156	172·3	86·1	1·935	—
7	3	231 255 265	250	83·4	1·921	—
10	10	825 768 843	812	81·2	1·910	—

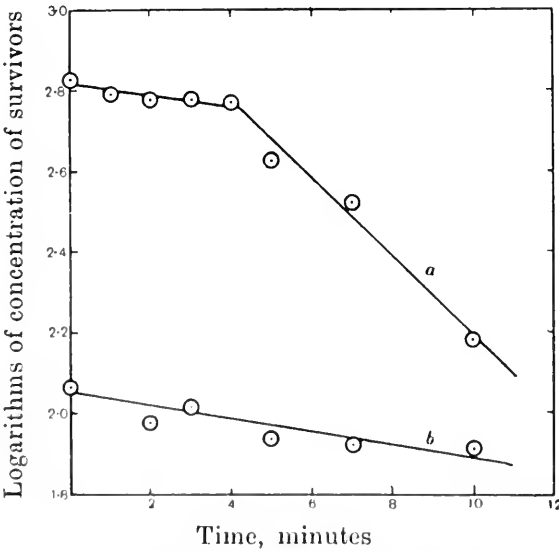


Fig. 6. Disinfection of *Staphylococcus pyogenes aureus* with 0·6 % phenol at 20° C. (Table IX.)
(a) 24 hours' culture at 37° C. (b) 24 hours' culture at 42° C.

(2) *General conclusions upon disinfection with phenol.*

Disinfection with phenol may in general be considered to proceed in accordance with a logarithmic law so that the concentration of survivors varies logarithmically with time, or, in other words, the rate of disinfection at any moment is proportional to the concentration of surviving bacteria $\left(-\frac{dn}{dt} = Kn\right)$.

Of the species worked with, anthrax spores, *B. typhosus*, and cultures of *B. paratyphosus* obtained by successive sub-culturing after short periods of growth, may be cited as proof of this assertion, without further remark. In my opinion, *B. coli commune* and *Staphylococcus pyogenes aureus* (if we allow for a short "period of lag" at the beginning of disinfection) may be added to this list.

Eijkman, however, came to the conclusion that the rate of disinfection of *B. coli commune* by phenol (1909) or hot water (1908) was not proportional to the concentration of survivors, except possibly for a short period in the middle of the disinfection, and that a much slower rate obtained not only at the beginning but also at the end. The "period of lag" constantly present at the start was called by him a "period of incubation." This is contrary to my own experience of *B. coli*, which, whether disinfected by phenol or by heat (see below), I have found to die at a rate approximately proportional to the concentration of survivors. Slight discrepancies occur at the beginning of disinfection, but these, I think, are attributed to error in sampling. My experience confirms that of Eijkmann (1908, p. 17, and 1909, p. 6), who had great difficulty in obtaining uniformity in cultures of *B. coli*.

In the case of *Staphylococcus* and 24 hours' cultures of *B. paratyphosus* the departure from the logarithmic law at the beginning of disinfection is of a different character. The *invariable* "incubation period" in the first case, and the *invariable* preliminary rush in the second, are evidently realities and must be attributed to some idiosyncrasy of the species in question. With *Staphylococcus*, after about 5 minutes, disinfection proceeds in an orderly manner so that the delay is possibly due to a slow permeability of the bacterial envelope to phenol.

In the case of 24 hours' cultures of *B. paratyphosus* (see H. C. 1908, p. 109) the surviving bacteria decrease in number more rapidly at first than would be the case if the rate of disinfection were simply proportional to the concentration of bacteria at the moment, and a suggested

explanation has already been put forward. This explanation has been criticised in the meantime by Hewlett (1909), so that a few further remarks may be permitted. The departure from the logarithmic law was found to be minimised by experimenting with material obtained by successively sub-culturing a small amount of a broth culture into fresh broth every 2—3 hours; in this case disinfection proceeded in a more regular manner and at the slower rate. This is shown in Fig. 7, which illustrates two experiments with *B. paratyphosus* and 0.6% phenol (*loc. cit.* Tables VII and XII), one made with a 24 hours' culture and the other with material which had been successively sub-cultured three times at three hours' intervals. In the former case logarithms of concentration of living bacteria, plotted against time, give a curve; in the latter case, however, disinfection is more nearly logarithmic throughout, the experimental points lie on a straight line and the velocity constant has an approximately constant value.

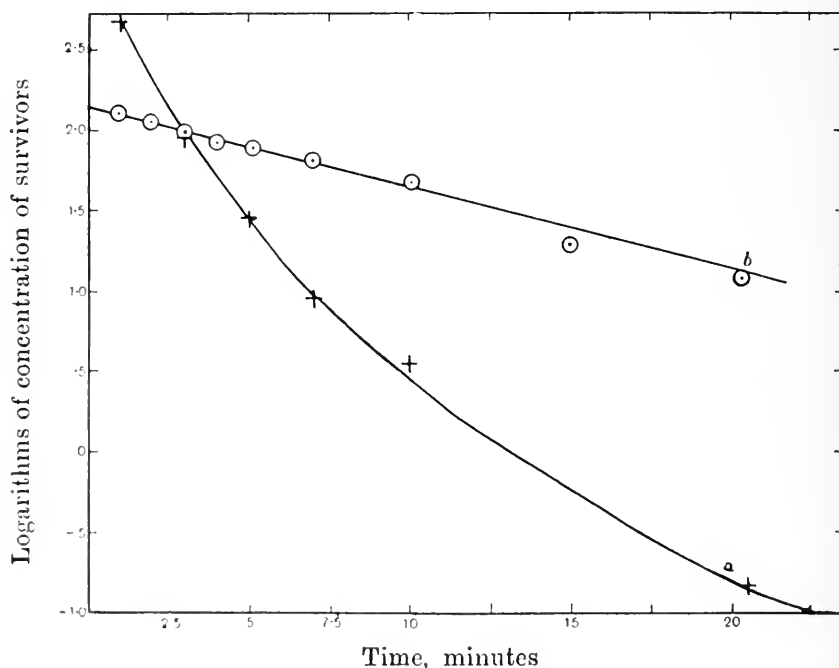


Fig. 7. (a) Disinfection of 24 hours' culture of *B. paratyphosus* with 0.6% phenol at 21° C. (H. C. 1908, Table VII.)

(b) Disinfection of 3 hours' culture (3rd generation of 3 hours' cultures) of *B. paratyphosus* with 0.6% phenol at 20° C. (H. C. 1908, Table XII.)

Hewlett (*loc. cit.* p. 50), after an analysis, among others, of the tables from which these curves are drawn, "can only see a slight balance in favour of the 'young' cultures giving a constant less variable than the constant obtained with 24 hours' cultures." Hewlett's treatment of

These data is not quite legitimate. The tables he compares are not strictly comparable, the times at which samples were taken being different in the different cases. Comparison of curves, on the other hand, in which logarithms of concentration of surviving bacteria are plotted against time, is unexceptionable. From experiments with 24 hours' cultures of *B. paratyphosus* a series of curved lines are obtained, whereas from experiments with successively cultured material the lines are approximately straight, see Fig. 7.

The following interpretation was suggested to account for this apparent anomaly. Whereas in the case of anthrax spores and other species, where disinfection proceeds logarithmically, we may suppose temporary differences in resistance to disinfectants to exist among the individual bacteria (see also pp. 240 and 282), in 24 hours' cultures of *B. paratyphosus* we must suppose in addition the existence of permanent differences in resistance among the individuals, these differences to be in some way bound up with the "age" of the culture.

In my previous paper this was perhaps not very satisfactorily expressed, which may account for some misunderstanding. The age of individuals, as long as multiplication is regularly maintained during growth of a culture, would probably be the same in all cultures of whatever history, provided the temperature of incubation were the same. But a difference might exist between the population of a 24 hours' and a 24 hours' culture, owing to the fact that in the latter case the culture medium had altered in composition by the time the last multiplication had taken place, as a result of the large amount of growth it had already sustained¹. In such a sense one may imagine differences to exist between "old" and "young" cultures. Whatever may be the correct manner of expression, it seems that the majority of the individuals in 24 hours' cultures of *B. paratyphosus* have a permanently weakened resistance to disinfectants, which must be distinguished from, and which they possess in addition to, those temporary differences in resistance by which I account for the logarithmic nature of disinfection in the ideal case.

(3) *Summary of Section II.*

1. Further experimental evidence is adduced to show that disinfection with phenol proceeds in accordance with a logarithmic law,

¹ In my experiments 24 hours' cultures would seem to have attained the limit of growth, from the approximately constant number of bacteria they contained.

in a manner analogous to that of a reaction of the first order, so that the velocity of disinfection at any moment is proportional to the concentration of surviving bacteria $\left(-\frac{dn}{dt} = Kn\right)$.

Experiments have been made with

- (a) *B. typhosus*,
- (b) *B. coli commune*,
- (c) *Staphylococcus pyogenes aureus*,

and compared with those made with anthrax spores and *B. paratyphosus*, which have been published previously.

2. In the case of *Staphylococcus py. aur.* there is invariably a short period (4 minutes with 0.6 % phenol) at the beginning of disinfection, when the disinfectant has little or no effect, after which disinfection proceeds in the normal manner. The suggestion is made that it is due to time taken for penetration of the bacterial envelope by the disinfectant.

3. In the case of 24 hours' cultures of *B. paratyphosus*, where disinfection proceeds at first at a rate which is in excess of what it would be if proportional only to the concentration of survivors, the majority of the individuals possess a permanently lessened resistance to the disinfectant. It is suggested, in amplification of a suggestion put forward in a previous paper, that this may be caused by exhaustion of the medium in which the bacteria have been grown. Cultures produced by successive sub-culture after short intervals of time show approximate conformity to the logarithmic law.

III. THE PROCESS OF DISINFECTION BY HOT WATER.

(1) *General remarks upon disinfection by heat.*

In disinfection by heat it is possible that three entirely different processes may be at work:

- (1) the direct effect of heat upon the proteins of the bacteria,
- (2) the effect, possibly hydrolytic, of water upon these proteins at high temperatures,
- (3) desiccation of the organisms.

In sterilisation of bacteria by dry heat a combination of (1) with (2) or possibly with (2) and (3) may occur. The germicidal action of hot water at temperatures of 45° C. to 55° C., however, presents a very suitable subject for the study of the simplest disinfection process. For, from the analogous behaviour of proteins and enzymes, when treated with

hot water, it would appear to present an uncomplicated instance of the second effect.

Madsen and Nyman (1907) investigated the rate at which anthrax spores dried in the air upon the surface of garnets (method of Krönig and Paul, 1897) were killed when placed in glass tubes and immersed in an oil bath at 100°C . and 110°C . respectively. At intervals one tube of garnets was removed, the garnets shaken in a measured quantity of water and plate cultures made. Their observations do not afford convincing evidence that, under these conditions, disinfection proceeds in accordance with a logarithmic law, but the authors, considering the roughness of the experimental method, seem satisfied with the agreement between calculated and observed values.

The following experiments refer to the effect of water at temperatures from 45°C . to 55°C ., this method of experiment having been adopted in order to avoid some of the complications mentioned above.

(2) *Method employed.*

A wide mouthed test tube, capacity about 50 c.c., was fitted with a cork having two holes, into each of which a small piece of glass tubing had been fitted. The smaller glass tube A (see Fig. 8) acted as a bearing for the stirrer C which was attached to a bicycle hub by a

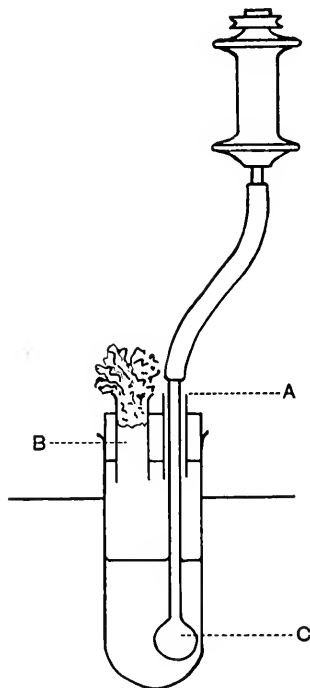


Fig. 8.

piece of india-rubber tubing. The bicycle hub had a small pulley at its upper end round which a cord passed to the shafting. The second tube, *B*, closed with a cotton wool plug, was used for adding the bacteria and for the withdrawal of the samples.

The whole apparatus being previously sterilised, 20 c.c. of sterile distilled water was placed in the tube and the whole allowed to remain in the bath sufficiently long to acquire the necessary temperature. A 24 hours' broth culture of the particular organism, grown at 37° C., was diluted about five to ten times, and at a given moment a small quantity of the diluted culture was introduced through the small tube *B*. At suitable times samples were withdrawn with sterilised pipettes, placed in separate small sterilised tubes and allowed to cool immediately.

At the conclusion of the experiment, plates were poured with measured quantities of the liquid in the series of tubes, smaller quantities (0.02 c.c.) from the earlier samples and larger quantities (0.2 to 0.4 c.c.) from the later samples.

The experiments were preferably so arranged that the disinfection tube contained about 500 to 2000 organisms per standard drop (25,000 to 100,000 per c.c.) at the start and samples were usually taken until there were less than 10 per drop.

The character of the disinfection process and its velocity at different temperatures was studied in the case of the following organisms :

- (a) *B. typhosus*,
- (b) *B. coli commune*,
- (c) *B. paratyphosus*,
- (d) *Staphylococcus pyogenes albus* and *Staphylococcus pyogenes aureus*,
- (e) *B. pestis*.

(a) *Experiments with B. typhosus.*

Experiments with 24 hours' cultures of *B. typhosus* were made over a series of temperatures from 49° C. to 54° C. The results of two experiments at 54.15° C. and 49° C. respectively are given in Table X and graphically expressed in Fig. 9; the results of two further experiments at 52° C. and 52.1° C. are given in Table XI.

Disinfection proceeded according to the usual rule, approximately constant values were obtained for the velocity constant $K \left(= \frac{1}{t_n - t_0} \log \frac{N_0}{N_n} \right)$ and approximately straight lines were obtained on plotting

time against logarithms of numbers of survivors present in unit volume. The first enumeration in the experiment at 49° C. (2008 bacteria per drop at 0·28 minutes) is out of line with the other determinations, and has been disregarded on the ground that, owing to imperfect mixing in so short a time, much stress should not be laid upon the result of the analysis of a sample taken during the first 20 seconds after addition of the bacteria. If this determination is included and used in the calculation of the values for the velocity constant, disinfection will apparently proceed at an excessive rate during the first minute ($K = \cdot 308$). For the reason given above it is hard to say whether the phenomenon is a reality or not. In other experiments there is, however, also a fall, though slight, in the value of K as disinfection proceeds, see Exp. II, Table X, and Exps. I and II, Table XI.

TABLE X.

Disinfection of B. typhosus with hot water.

Exp. I, 9. 6. '09. Temperature 49° C.

Time, minutes = t	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0·28	1	1881 2122 2022	2008	2008	3·303	—
1·0 = t_0	1	1298 1098	1198	1198 = N_0	3·078	—
2·05	1	914 769 1093	925	925	2·966	·107
3	3	1782 2246 2770	2266	755	2·878	·100
4	4	1947 2522 2038	2169	542	2·734	·114
5	$\left\{ \begin{array}{l} 1 \\ 10 \end{array} \right.$	$\left\{ \begin{array}{l} 449 \\ 5180 \ 4640 \end{array} \right.$	$\left\{ \begin{array}{l} — \\ — \end{array} \right.$	488	2·688	·097
7	$\left\{ \begin{array}{l} 6 \\ 10 \end{array} \right.$	$\left\{ \begin{array}{l} 1788 \\ 2800 \ 2940 \end{array} \right.$	$\left\{ \begin{array}{l} — \\ — \end{array} \right.$	289	2·461	·103
10	10	1113 1067 1204	1128	112·8	2·052	·114
15	$\left\{ \begin{array}{l} 10 \\ 20 \end{array} \right.$	$\left\{ \begin{array}{l} 211 \\ 514 \end{array} \right.$	$\left\{ \begin{array}{l} — \\ — \end{array} \right.$	24·2	1·384	·113
20	$\left\{ \begin{array}{l} 9 \\ 10 \\ 20 \end{array} \right.$	$\left\{ \begin{array}{l} 30 \\ 28 \\ 51 \end{array} \right.$	$\left\{ \begin{array}{l} — \\ — \\ — \end{array} \right.$	3	·477	·137
Mean						·111

EXP. II, 9. 6. '09. Temperature 54.15° C.

0·25	1	509	444	472	475	$475 = N_0$	2·677	—
0·5	1		198	201	199·5	199·5	2·300	1·51
1·0	3	121	86	108	105	35	1·544	1·51
1·5	5	40	54	46	46·6	9·3	·968	1·37
2·0	10	16	18	13	15·66	1·566	·195	1·42
							Mean	1·45

TABLE XIII.

Disinfection of B. coli commune with hot water.

Exp. I, 26. 2. '10. Temperature 49° C.

Time, minutes = t	Amount of sample taken, drops	Numbers counted on plates (gelatine)			Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0.75 = t_0	1	341	375	276	331	331 = N_0	2.520	—
1.5	1	313	307	332	317	317	2.501	.025
2.5	1	249	282	295	275	275	2.439	.046
4	2	500	534	462	499	249.5	2.397	.038
6	5	922	868	834	875	175	2.243	.053
9	10	1740	1710	1490	1650	165	2.217	.037
14	20	1440	1540	1290	1420	71	1.851	.050
20	20	832	836	704	791	39.5	1.597	.048
Mean								.042

Exp. II, 26. 2. '10. Temperature 52.5° C.

0.25 = t_0	1	388	443	374	402	402 = N_0	2.604	—
0.75	1	360	411	388	386	386	2.587	.034
1.5	1	309	290	268	289	289	2.461	.114
2.5	2	478	469	493	480	240	2.380	.099
4	5	617	593	598	602.7	120.5	2.081	.139
6	$\left\{ \begin{array}{l} 13 \\ 20 \end{array} \right.$	812		$\left\{ \begin{array}{l} — \\ — \end{array} \right.$		63.8	1.805	.139
		1260	1310					
8	20	574	647	697	639	32	1.505	.142
Mean								.111

in Exp. II and in both experiments in Table XIII to increase as disinfection proceeds. The latter type bears a resemblance to the experiments of Eijkman (see above, p. 249). All, however, conform more nearly than his to what I may call the ideal case, and, considering that the deviation is small, and not consistently in one direction, and that the species is moreover extremely variable, I conclude that here also disinfection proceeds so that the rate is approximately proportional to the concentration of survivors, and that straight lines most satisfactorily express the relation between the experimental points when logarithms of the latter are plotted against time.

The variability of this species is well shown by a comparison of Tables XII and XIII, where rate of disinfection of apparently similar material, at approximately the same temperature, is in the one case about four times as great as in the other.

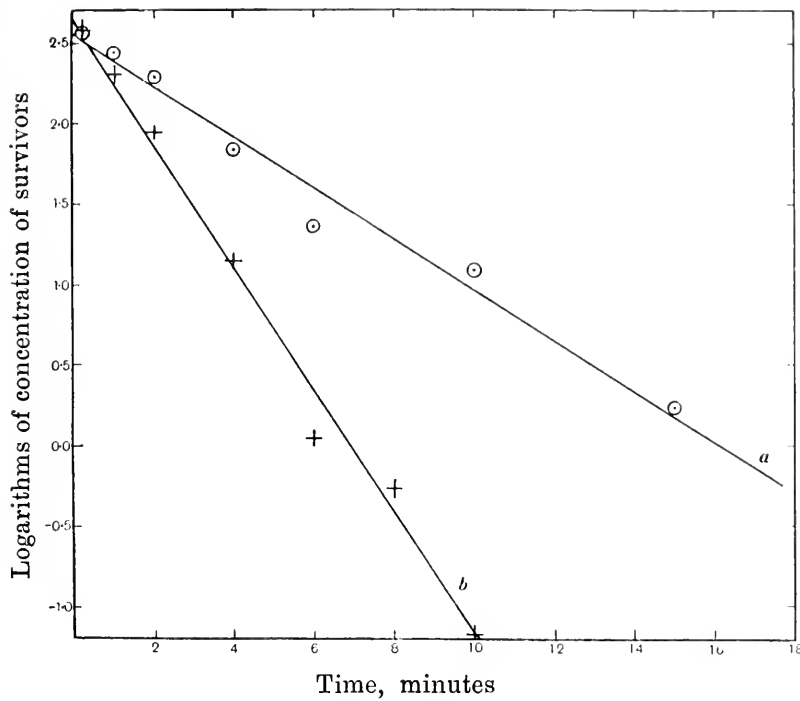


Fig. 10. Disinfection of *B. coli commune* with hot water (Table XII).
 (a) Exp. I at 48.9° C. (b) Exp. II at 52.7° C.

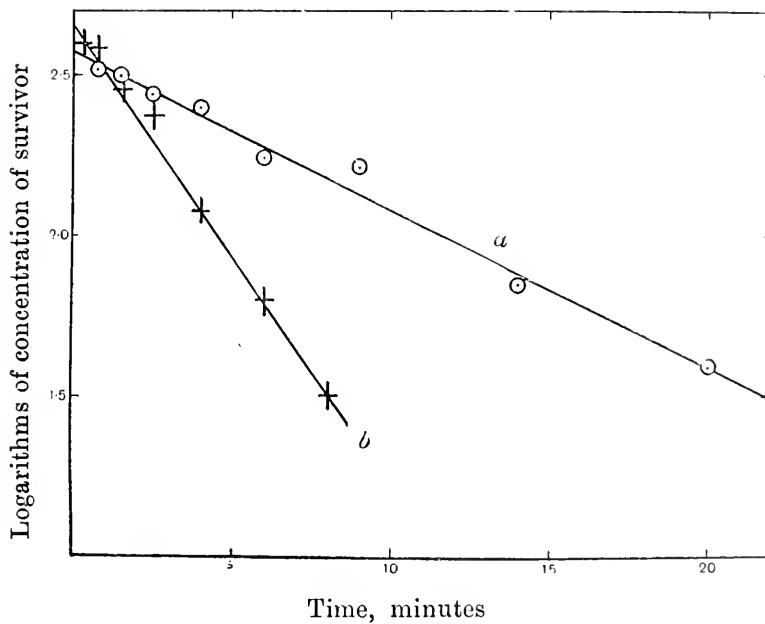


Fig. 11. Disinfection of *B. coli commune* with hot water (Table XIII).
 (a) Exp. I at 49° C. (b) Exp. II at 52.5° C.

After the first few minutes the accordance with the logarithmic law is very good, but, as was found to be the case with chemical disinfectants, the reaction proceeds more rapidly at first than would be the case were the rate dependent only upon the concentration of surviving bacteria.

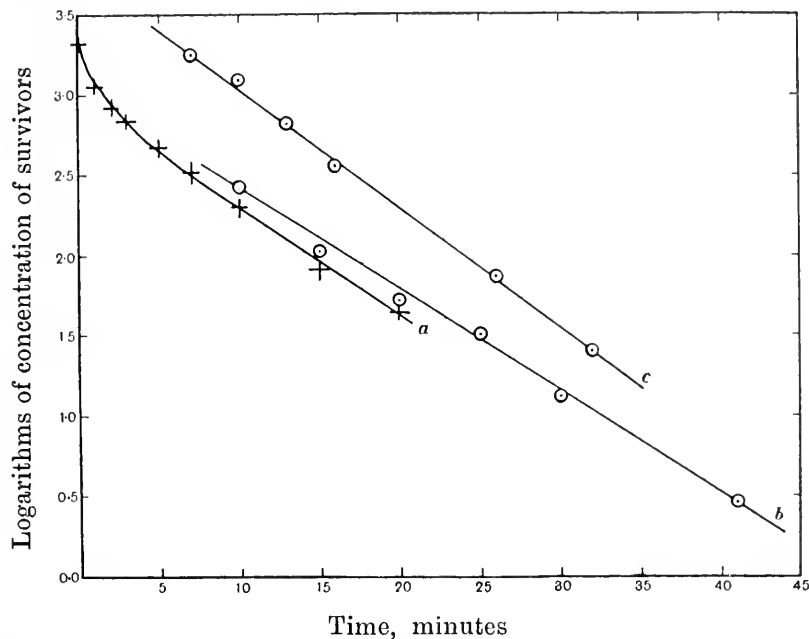


Fig. 12. Disinfection of *B. paratyphosus* with hot water (Table XIV).
(a) Exp. I at 47.2° C. (b) Exp. II at 47.2° C. (c) Exp. III at 47.35° C.

(d) *Staphylococcus pyogenes albus* and *Staphylococcus pyogenes aureus*.

Staphylococcus pyogenes albus. The results of two experiments with *Staphylococcus albus* at 49° C. and 53.05° C. respectively are given in Table XV and Fig. 13; they show fair agreement with the logarithmic law.

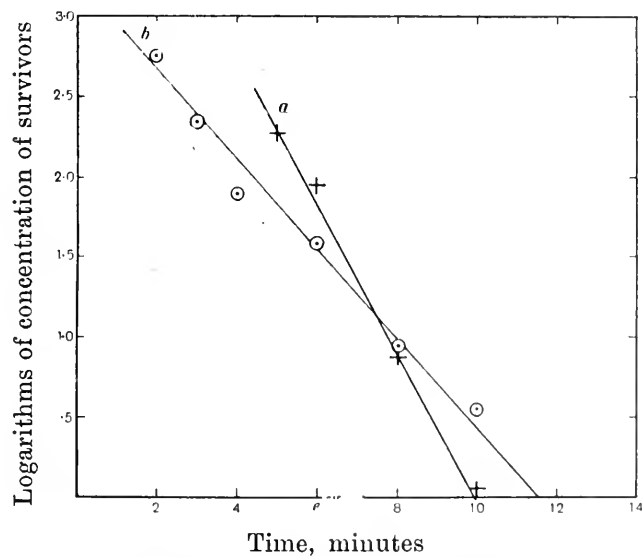


Fig. 13. Disinfection of *Staphylococcus pyogenes albus* with hot water (Table XV).
(a) Exp. I at 53.05° C. (b) Exp. II. at 49° C.

(*Staphylococcus pyogenes aureus*. On examination of the results of experiments with *Staphylococcus pyogenes aureus* (Tables XVI to XIX and Figs. 14 to 17), it is seen that in this case disinfection by hot water conforms to no constant type. In almost all cases the latter part of the disinfection proceeds logarithmically (see Exps. I and II, Table XIX, and Fig. 17, which are concerned with the course of disinfection after 10 minutes and 16 minutes respectively have elapsed), but the time relations of the disinfection process as a whole present great variety among which three distinct types can be distinguished:

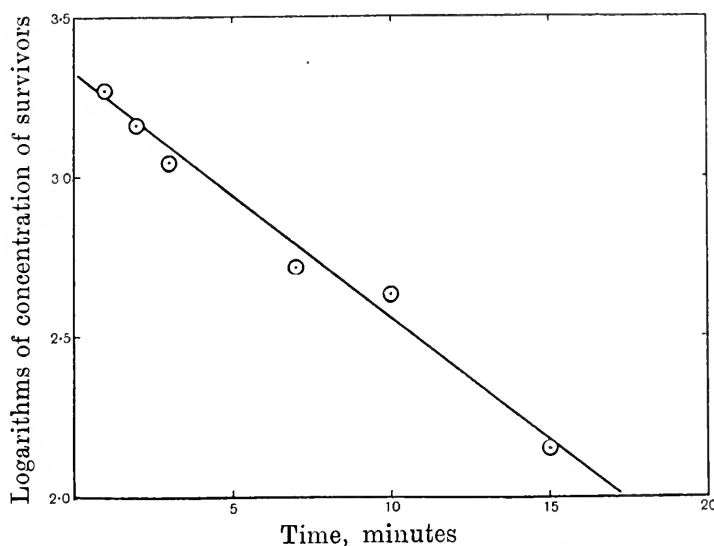


Fig. 14. Disinfection of *Staphylococcus pyogenes aureus* with hot water at 49.3°C . (Table XVI).

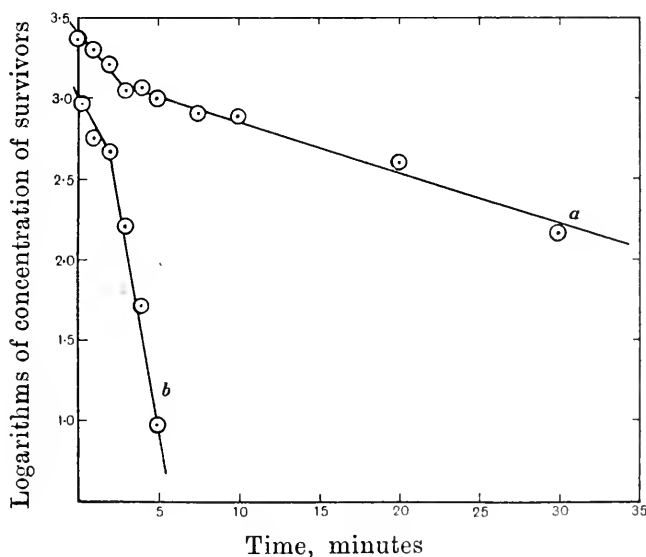


Fig. 15. Disinfection of *Staphylococcus pyogenes aureus* with hot water (Table XVII).
 (a) Exp. I at 49.3°C . (b) Exp. II at 49°C .

TABLE XVII.

Disinfection of Staphylococcus pyogenes aureus with hot water.

EXP. I, 13. 5. '09. (24 hours' culture from stock culture 36 days old.) Temp. 49·3° C.

Time, minutes = t	Amount of sample taken, drops	Numbers counted on plates (agar)			Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$1 = t_0$	1	2168	2522	2024	2238	$2238 = N_0$	3·350	—
2	1	1698	1649	1516	1621	1621	3·210	·140
3	1	1105	1182	1119	1135	1135	3·055	·147
4	1	1068	1343	1125	1179	1179	3·072	·093
5	1	971	1023	1082	1025	1025	3·011	·085
7	1		819		819	819	2·913	·073
10	2	1372	1558	1619	1516	758	2·880	·052
20	3	1320	1160		1240	413	2·616	·039
30	7		1101		—	146	2·164	·041
	10		1388					

EXP. II, 3. 6. '09. (24 hours' culture from stock culture 13 days old.) Temp. 49° C.

0·25 = t_0	1		912		912	$912 = N_0$	2·960	—
1·0	1	525	570		547	547	2·738	·30
2	1	542	393		467	467	2·669	·17
3	1	224	108		166	166	2·220	·27
4	1	42	60		51	51	1·708	·33
5	2	22	14	20	18·66	9·33	·970	·42
Mean								·30

(1) Disinfection rate is throughout approximately proportional to the concentration of survivors (Table XVI), and a straight line best expresses the relation of the points when logarithms of survivors are plotted against time (Fig. 14).

(2) There is a "period of lag" at the beginning of disinfection (see Exp. II, Table XVII, and Exps. I, II and III, Table XVIII, where the value of K progressively increases) recalling disinfection of this species with phenol; a convex curve is obtained when logarithms of concentration of survivors are plotted against time (Fig. 15 *b* and Fig. 16).

(3) Disinfection is much accelerated at the beginning and becomes slower afterwards (see Exp. I, Table XVII, where the value of K progressively decreases); a concave curve is obtained when logarithms of concentration of survivors are plotted against time (see Fig. 15 *a*).

TABLE XVIII.

Disinfection of Staphylococcus pyogenes aureus by hot water.

(24 hours' culture from very young stock cultures.)

EXP. I, 24. 1. '10. (24 hours' culture from stock culture 5 days old.) Temp. 48·9° C.

Time, minutes = t	Amount of sample taken, drops	Numbers counted on plates (gelatine)			Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0·25 = t_0	1	1800	1790	1090	1560	1560 = N_0	3·193	—
1·0	1	1230	1330		1280	1280	3·107	Mean ·0486 } ·0491 ·0434 ·0532 ·127
	2	1890	1930	2010	1940	970	2·987	
	2	980	910		945	472·5	2·674	
	{ 7	29			—	4·8	·681	
	{ 10	52			—			

EXP. II, 24. 1. '10. (24 hours' culture from stock culture 6 days old.) Temp. 52·9° C.

0·25 = t_0	1	1230	1110	1180	1170	1170 = N_0	3·068	—
1·0	1	990	840	810	880	880	2·944	Mean ·188 } ·165 ·176 ·223 ·380
	2	1020	1270		1145	572	2·757	
	{ 6	590			—	102	2·009	
	{ 10	1130	940		—			
	10	7	0	0	2·3	0·23	— ·638	

EXP. III, 9. 2. '10. (24 hours' culture from stock culture 2 days old.) Temp. 52·6° C.

0·25	1	Lost						
1·0 = t_0	1	517	594		555·5	555·5 = N_0	2·745	—
	1	353			353	353	2·548	Mean ·223 } ·197 ·228 ·243 ·322 ·344
	2	264	195		229·5	114·7	2·060	
	5	182	151	176	169·7	33·9	1·530	
	{ 8	29			—	3·1	·491	
	{ 10	26			—			
	20	4			4	0·2	— ·699	

The reason¹ for this irregularity was not discovered, it is possible that the cause lies in the variability of the species and may depend on the special character of the stock culture from which the 24 hours' culture was prepared. It was generally true that disinfection of cultures

¹ The suggestion is made that in some cases these irregularities are only apparent, and may be due to agglutinations, taking place at once (type 3), or after an interval of time (type 2), when an emulsion of the bacteria is suspended in hot water. Cultures of *Staphylococcus* readily agglutinate when suspensions in water or broth are heated to temperatures somewhat higher than those employed in the present experiments. Attempts were therefore made to trace microscopically any agglutination that might occur, but nothing significant could be detected. It is however certain that an agglutination which would apparently increase the rate of disinfection three or four times (see Tables XVII and XVIII) would be very insignificant and almost impossible to detect with the microscope.

made from fresh stock cultures (under a week old) was of the 2nd type, while that of material obtained from older stock cultures conformed to the 1st or 3rd type.

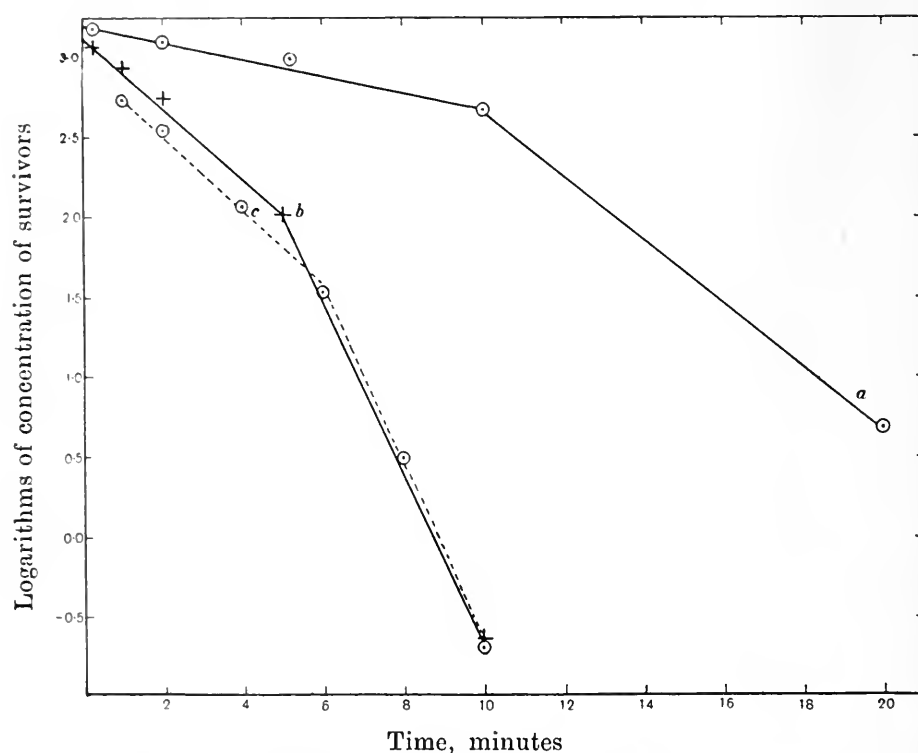


Fig. 16. Disinfection of *Staphylococcus pyogenes aureus* with hot water (Table XVIII). (a) Exp. I at 48.9° C. (○). (b) Exp. II at 52.9° C. (+). (c) Exp. III at 52.6° C. (○).

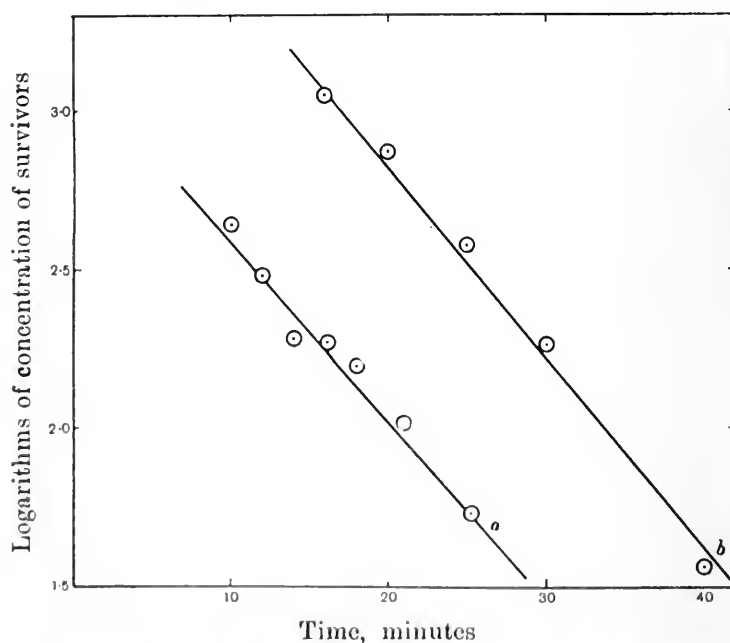


Fig. 17. Disinfection of *Staphylococcus pyogenes aureus* by hot water at 49.3° C. (Table XIX).

(a) Exp. I. (b) Exp. II.

TABLE XIX.

Disinfection of Staphylococcus pyogenes aureus with hot water at 49.3° C.

Exp. I, 8. 4. '09. (24 hours' culture from stock culture 11 days old.)

Time, minutes = t	Amount of sample taken, drops	Numbers counted on plates (agar)			Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
10 = t_0	1	458	372	489	439.7	439.7 = N_0	2.643	—
12	1	332	271	306	303	303	2.481	.081
14	2	441	369	341	384	192	2.283	.090
16.1	3	555	527	598	560	187	2.272	.061
18	5	821	788	755	788	158	2.199	.055
21	4	409			—	107.8	2.033	.055
	5	598	608		—			
	10	972			—			
25.25	10	450	488	666	535	53.5	1.728	.060
Mean								.067

Exp. II, 14. 4. '09. (24 hours' culture from stock culture 7 days old.)

16 = t_0	$\left\{ \begin{array}{l} 1 \\ 2 \end{array} \right.$	$\left\{ \begin{array}{l} 1153 \\ 2109 \end{array} \right.$	$\left\{ \begin{array}{l} 1243 \\ \end{array} \right.$	$\left\{ \begin{array}{l} \text{—} \\ \text{—} \end{array} \right.$		1126 = N_0	3.051	—
20	3	2158	2207	2352	2239	746	2.873	.044
25	5	1976	1846	1845	1889	378	2.577	.053
30	10	1818	1837	1803	1819	181.9	2.260	.056
40	10	305	412	373	363	36.3	1.560	.062
Mean								.054

Besides a general want of consistency in the character of the disinfection process itself, the average velocity of disinfection under apparently similar conditions is also extremely variable. Great difficulty in obtaining constancy of resistance in cultures would appear to be characteristic of work with *Staphylococcus*. In disinfection by hot water it was unusual to obtain the same result twice, unlooked-for alterations in resistance were constantly occurring, for which the responsibility was not satisfactorily traced. For example, Table XVII, Experiment II, shows a value of the velocity constant which is nearly three times that of the average in Experiment I, and four to five times that in Experiments I and II, Table XIX, all of which were made under almost identical conditions as regards temperature.

There is no entirely satisfactory explanation for these inconsistencies. As mentioned above, I find that the previous history of cultures of *Staphylococcus* modifies very considerably their behaviour towards

disinfectants; in the case of disinfection with phenol, difference in temperature of incubation had an unexpectedly great influence upon the resistance of the bacteria towards phenol (see above, p. 247). It is probable that it also modifies its resistance to hot water.

(e) *B. pestis*.

The results of one experiment with *B. pestis* at 50° C. (Table XX and Fig. 18) show fair agreement with the logarithmic law.

TABLE XX.

Disinfection of B. pestis with hot water at 50° C.

Time, minutes = t	No. of organisms in 5 drops disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
4.25 = t_0	9440 = N_0	3.975	—
7.0	6880	3.838	.050
9.75	6160	3.790	.034
13.0	4460	3.649	.037
16.0	3340	3.524	.038
			Mean .040

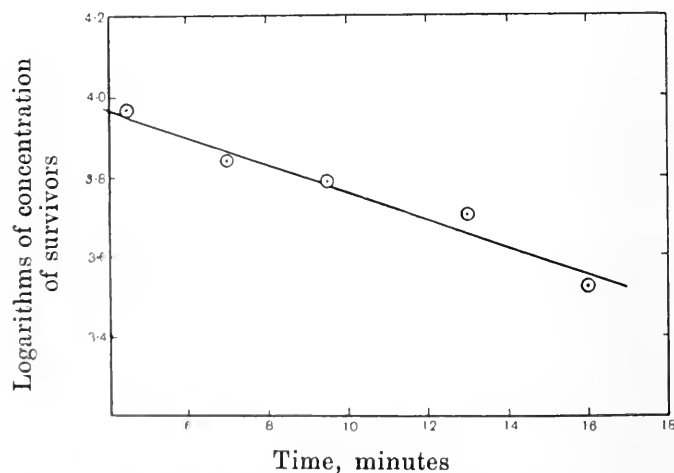


Fig. 18. Disinfection of *B. pestis* with hot water at 50° C. (Table XX).

(3) *Temperature coefficient of disinfection by hot water.*

B. typhosus. The experiments with *B. typhosus* extended over a range of 5° C., and the temperature coefficient of disinfection by hot water could therefore be determined with some accuracy by comparing

velocity constants of two comparable disinfections at different temperatures. In Experiments I and II, Table X, the mean value of the velocity constant at 54.1°C. was 1.45, while that corresponding to 49°C. was 0.111. This gives an increase in reaction velocity of 13.1 times for 5°C. rise in temperature, *i.e.* 1.67 times per 1°C. , and about 170 times per 10°C. rise in temperature, if the temperature effect is assumed to be consistent.

To ascertain the truth or otherwise of this assumption and for purposes of comparison, some additional experiments were made with *B. typhosus*, using an end-point method (see H. C. 1908, p. 118). Test tubes containing 5 c.c. of distilled water and fitted with standardised capillary pipettes were placed in thermostats maintained at different temperatures and sown with a small quantity (5 standard drops = 0.1 c.c.) from a 24 hours' culture of *B. typhosus*. From time to time samples (4 drops = 0.08 c.c.) were withdrawn by means of the capillary pipette and added to tubes of glucose broth, which were then incubated. The time necessary for complete disinfection was taken as the mean between that of the last positive and first negative test culture. These times of "complete disinfection" strictly mean the time taken for an initial concentration of about 5,000,000 bacteria per c.c. to be reduced to less than 12 per c.c., or till there may not be one living bacteria present in the sample taken, 0.08 c.c.

The results of two experiments (Table XXI) are consistent one with the other. A logarithmic relation was found to express the effect of temperature upon disinfection velocity, as was expected from analogy with other cases of disinfection which have been investigated. Logarithms of times taken for disinfection (reciprocals of mean reaction velocity) were found to be proportional to the differences of temperature, and, when plotted one against the other, gave approximately a straight line, as in Fig. 19.

Whether rise of temperature affects the velocity of disinfection by the same law in a simple logarithmic manner or in accordance with the law of Arrhenius, it is impossible to say, for within a small range of temperature the two become almost identical. The somewhat rough experimental method could not be expected to give very strict mathematical agreement with either, though as a matter of fact satisfactory agreement with both is obtained (see also temperature coefficient of disinfection with disinfectants, H. C. 1908, p. 153).

In any case the effect of temperature upon disinfection with hot water is seen to be consistent and very great. From Table XXI the mean

value 1.60 is obtained as temperature coefficient per 1° C. From the slope of the smoothed curve in Fig. 19 it is seen that a rise of 10° C. in temperature from 49° C. to 59° C. indicates an increase of 2.00 in the logarithms of the times taken, *i.e.* an increase in the mean velocity of disinfection of 100 times, or 1.58 times for 1° C. These values are in fair agreement with that obtained by comparing velocity constants, *viz.* 1.67 per 1° C.

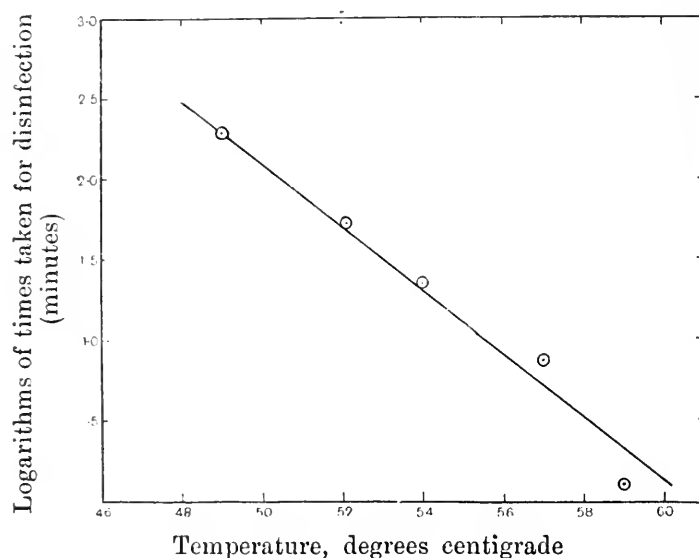


Fig. 19. Effect of different temperatures upon disinfection of *B. typhosus* by hot water (Table XXI).

TABLE XXI.

Temperature coefficient of disinfection of B. typhosus with hot water (end-point method).

	Temp. of exp., ° C.	Time taken for disinfection*, minutes = <i>t</i>	Log ₁₀ <i>t</i>	Log ₁₀ (temperature coefficient for 1° C.)	Temperature coefficient per 1° C.
EXP. I, 10. 6. '09	59	1.26	.100	.250	1.78
	54	22.5	1.352		
	49	196	2.292		
EXP. II, 4. 6. '09	57	7.5	.875	.173	1.46
	52.1	53	1.724		
				Mean	1.60

* Strictly speaking, reduction of about 5,000,000 per c.c. to less than about 12 per c.c.

B. coli commune and *Staphylococcus pyogenes aureus*.

In the preceding experiments with *B. coli commune* and *Staph. py. aur.* there are a few sets which can be used for calculation of the temperature coefficient of disinfection by hot water, assuming the effect of temperature to be consistent and in accordance with a logarithmic law. These are collected in Table XXII. Both species are extremely variable, and it is necessary, even more than was the case with *B. typhosus*, that for comparison of disinfection rate at different temperatures the experiments should be carried out on the same day, with the same material, etc. In Table XXII, for example, it will be seen that whereas on two occasions velocity constants obtained for the disinfection of *B. coli* at practically the same temperatures are very different, the temperature coefficients reckoned from them are very similar, 1·23 and 1·32 per 1° C.

TABLE XXII.

Temperature coefficient of disinfection by hot water, B. coli commune and Staphylococcus pyogenes aureus.

	Organism	Temp., ° C.	Difference in temp., ° C.	Mean value of velocity constant, K	Temp. coefficient per 1° C.
Exp. 15. 2. '10 (Table XII)	<i>B. coli commune</i>	48·9	3·8	·172	1·23
	„	52·7		·381	
Exp. 26. 2. '10 (Table XIII)	<i>B. coli commune</i>	49	3·5	·0424	1·32
	„	52·5		·111	
Exp. 24. 1. '10 (Table XVIII)	<i>Staph. py. aur.</i>	48·9	4·0	·0486	1·40
	„	52·9		·188	

The temperature coefficient of disinfection with hot water considerably exceeds that of disinfection by other means. Disinfection of *B. paratyphosus* with phenol and other coal-tar disinfectants was found to have a temperature coefficient of 10 per 10° C. under certain conditions, while with metallic salts the figure was very much lower and approximated to that usually obtaining in chemical reactions. Disinfection by drying has also a low temperature coefficient, viz. 2 to 3 for 10° C. (Paul, 1909 and Paul, Birstein and Reuss, 1910). In the case of disinfection by hot water we have figures ranging from about 136 per 10° C. (1·635 per 1° C., if we take the mean of the values obtained by the two different methods) in case of *B. typhosus*, to 29 per 10° C. (1·40 per 1° C.) in case of *Staphylococcus* and 12 per 10° C. (1·28 per 1° C.) in case of *B. coli*.

The figures in cases like that of *B. coli* and *Staph. py. aur.*, where data are scanty, must only be considered approximate. The high temperature coefficient makes experiment impossible except over a very small range of temperature, and one can only obtain the effect for 10° C. by calculation; a small error, therefore, in the value obtained for 1° C. will lead to a large one when this value is raised to the 10th power to obtain the value for 10° C. rise in temperature.

(4) *Influence of acids and alkalis.*

The influence of reaction upon disinfection by hot water was noticed in the course of some experiments in which a suspension of *B. typhosus* in broth was the material used for investigation. The experiments were made in order to obtain information which might be of service in the manufacture of vaccines. The temperature was 54° C. The method of experiment was exactly as in the preceding experiments, except that, in place of distilled water, the bacteria were suspended in broth in which the same species had grown for 40 hours at 37° C. and which had been sterilised by filtration through a Berkefeld filter.

Disinfection was logarithmic, but the rate was much slower than in distilled water under the same conditions. This was found to be due to the slight alkalinity of the broth. On addition of a small quantity of acid (0.25 c.c. $\frac{N}{1}$ acetic acid to 20 c.c. broth) up to neutralisation point (litmus), the rate of disinfection was increased about 8 times, see Table XXIII, Experiments of 22. 7. '09 and Fig. 20. A small extra amount of acid, too small to exert any disinfectant action of itself¹ (in all 0.5 c.c. $\frac{N}{1}$ acetic acid added to 20 c.c. broth), rendered disinfection so quick that its rate could not be measured, see Table XXIV, Experiment II. In this case, the value of the velocity constant of disinfection

¹ Acetic acid in the presence of 1% peptone has been shown to exert a disinfectant action upon *B. coli commune* at and above a concentration of $\frac{N}{11}$ (Winslow and Lockridge, 1906). *B. typhosus* is probably more susceptible, but in the above case (0.25 c.c. $\frac{N}{1}$ acetic acid to 20.25 c.c. neutralised broth) it is likely that the acidity of the medium fell far short of $\frac{N}{100}$. The broth contained besides 1% peptone, a considerable proportion of meat extractives, which also, by combining with the acid added, would tend to further reduce the concentration of free acid in the medium.

TABLE XXIII.

Effect of small differences in reaction upon the disinfection of *B. typhosus* in broth at 54.1° C.

EXP. I, 22. 7. '09. Medium=filtered culture (40 hrs. at 37° C.) of *B. typhosus*.

Time, minutes = t	Amount of sample plated, drops	Numbers counted on plates (agar)			Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0.32 = t_0	1	2796	2888	2954	2879	2879 = N_0	3.459	—
1.07	1	1704	1982	1894	1860	1860	3.270	.25
2.15	2	1160	1029	1187	1125	562	2.750	.39
3.50	3	565	573	504	547.3	182.4	2.261	.38
6.00	{	5	157		—	38.1	1.581	.33
		9	339		—			
		10	418		—			
10.00	{	10	20	20	—	1.62	.209	.34
		20	25		—			
Mean								.34

EXP. II, 22. 7. '09. Medium=neutralised filtered culture (40 hrs. at 37° C.) of *B. typhosus* (10 drops=0.25 c.c. $\frac{N}{I}$ acetic acid added to 20 c.c. broth).

0.28 = t_0	1	590	602	679	624	624 = N_0	2.795	—
0.53	1	154	36	266	152	152	2.182	2.45
1.0	3	40	19	40	33	11	1.041	2.44
1.5	3	2	0	0	0.66	0.22	-.658	2.83
Mean								2.57

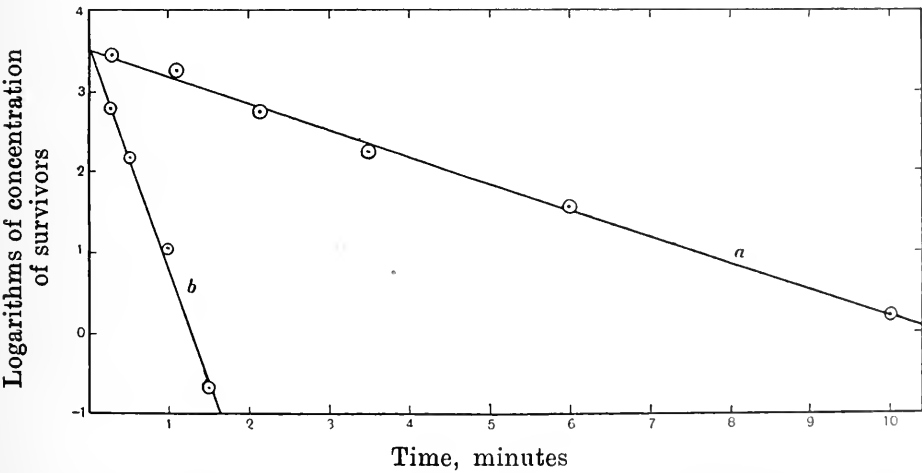


Fig. 20. Effect of reaction upon disinfection of *B. typhosus* in broth at 54.1° C. (Table XXIII).

- (a) Exp. I, broth slightly alkaline.
- (b) Exp. II, broth made just neutral (litmus) with acetic acid.

in the original broth was 0.44; in the faintly acid broth, where about 3000 organisms per drop were reduced to less than 1 per drop in 15 seconds, the value of the velocity constant was more than 17.6, i.e. more than 40 times as great as in the original.

TABLE XXIV.

Effect of small differences in reaction upon the disinfection of B. typhosus in broth at 54° C.

EXP. I, 24. 7. '09. Medium=filtered culture (40 hrs. at 37° C.) *B. typhosus*.

Time, minutes = t	Amount of sample plated, drops	Numbers counted on plates (agar)			Mean	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0.27 = t_0	1	2640	2816	3232	2896	2896 = N_0	3.462	—
0.98	1	2068	1420	1754	1747	1747	3.242	.31
2.00	1	165	240	325	243	243	2.386	.62
3.53	3	357	351		354	118	2.072	.43
6.17	5	59	40		49.5	9.9	.996	.42
10.27	{ 10	1	3		—	0.125	- .903	.44
	{ 20		1		—			
Mean								.44

EXP. II, 24. 7. '09. Medium=filtered culture (40 hrs. at 37° C.) *B. typhosus* + 20 drops (0.5 c.c.) $\frac{N}{1}$ acetic acid to 20 c.c. broth.

0.25 = t_0	1	0	0	0	—	—	—	More than
0.67	3	0	0	1	—	—	—	17.6*

* In Exp. II, disinfection after 0.25 minutes is more complete than after 10.27 minutes in Exp. I, i.e. disinfection rate is more than 40 times that of Exp. I, K =more than 17.6.

For the sake of comparison with the above experiments were made to investigate the influence of reaction upon disinfection in distilled water. In Tables XXV and XXVI are set forth the results of two comparable sets of experiments, in which *B. typhosus* was heated at 54° C. in water to which were added various small amounts of acid¹ and alkali insufficient in themselves to exert a disinfectant action. The smallest amounts which were employed were sufficient to render the solutions acid or alkaline to the extent of $\frac{1}{7000}$ th N respectively. In the case in which alkali was added, the solution was just alkaline to phenol phthalein. These small amounts were not without effect

¹ Death rate at 20° C. of *B. typhosus* suspended in water containing a concentration of $\text{H}_2\text{SO}_4 = \frac{N}{500}$ was found to be insignificant. Disinfection by H_2SO_4 may be assumed to have a low temperature coefficient from analogy with other mineral disinfectants.

upon the rate of disinfection by hot water, which in the case of alkali was increased about 1·5 to 2·0 times, and in the case of acid 5 to 7 times. On further addition of acid disinfection became too quick to study, whereas the increase in rate on adding more alkali was comparatively slight.

It therefore follows that, in the preparation of vaccines, no conclusion drawn from one set of experiments can be usefully applied to another set, unless the composition and reaction of the medium, in which the bacteria are heated, be kept strictly constant. This is almost impossible for, in the case of broth cultures, the reaction of the broth will, within small limits, depend upon the extent of the growth of organisms. It is not possible to control this, and small differences arising therefrom will have a very significant influence upon the rate of destruction by heat of the organisms suspended in the medium.

TABLE XXV.

*Effect of small amount of acid and alkali upon disinfection of
B. typhosus in distilled water at 54° C.*

EXP. 4. 8. '09. Distilled water.

Time, minutes = t	Amount of sample taken, drops	Numbers counted on plates	Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0·23 = t_0	1	3360 3700	3530 = N_0	3·548	—
0·55	1	2260 2240 2660	2390	3·378	·531
1·07	3	2780 2640	903	2·956	·705
2·5	{ 9 10	{ 1400 2370 1970 }	198	2·297	·551
Mean					·596
<hr/>					
$\frac{N}{7000}$ alkaline (NaOH)*.					
0·25 = t_0	1	1220 1380	1300 = N_0	3·114	—
0·90	1	245 183 160	196	2·292	1·26
2·05	{ 1 3	{ 11 49 39 }	14·1	1·149	1·09
Mean					1·17
<hr/>					
$\frac{N}{7000}$ acid (H_2SO_4).					
0·22 = t_0	{ 1 10	{ 294 2490 }	253 = N_0	2·403	—
0·51	{ 10 20	{ 217 296 }	17·1	1·233	4·03
0·92	{ 10 20	{ 111 114 }	7·5	·875	2·18
Mean					3·10

* Just alkaline to phenolphthalein.

TABLE XXVI.

*Effect of small amount of acid and alkali upon disinfection of
B. typhosus in distilled water at 52° C.*

EXP. I, 19. 8. '09. Distilled water.

Time, minutes = t	Amount of sample taken, drops	Numbers counted on plates			Mean no. of bacteria present in 1 drop disinfection mixture, = N	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0.33 = t_0	1	7250	5420	5350	6010 = N_0	3.779	1.65
1.0	3	7850	5500		2225	3.347	
<hr/>							
EXP. II.	$\frac{N}{7000}$	alkaline (NaOH)*.					
0.33 = t_0	2	6930	6230	7550	3450 = N_0	3.538	.95
1.0	15	11340			805	2.906	
	20	15260	17660				
<hr/>							
EXP. III.	$\frac{N}{3500}$	alkaline (NaOH).					
0.33 = t_0	5	3840	3780	3670	753 = N_0	2.877	1.26
1.0	16	1910			109	2.037	
	20	2020					
<hr/>							
EXP. IV.	$\frac{N}{1750}$	alkaline (NaOH).					
0.33 = t_0	10	6180	7860	8780	761 = N_0	2.881	1.26
1.0	19	1780			111	2.045	
	20	2540					
<hr/>							
EXP. V.	$\frac{N}{875}$	alkaline (NaOH).					
0.33 = t_0	16	9250			587 = N_0	2.769	1.31
1.0	20	11870					
	19	1600			78.5	1.895	
	20	1460					
<hr/>							
EXP. VI.	Distilled water.						
0.33 = t_0	1	930	1020	1130	1030 = N_0	3.013	.62
1.0	3	760	1280		393	2.594	
	5	2280					
<hr/>							
EXP. VII.	$\frac{N}{7000}$	acid (H_2SO_4).					
0.33 = t_0	5	179	179		35.8 = N_0	1.554	at least 4.29
1.0	20	(?) 2	0		(?) 0.05	(?) -1.301	

* Just alkaline to phenolphthalein.

(5) *Analogy between the disinfection of bacteria by hot water and the "heat coagulation" of proteins.*

The disinfection of bacteria by heat in presence of water exhibits a striking analogy with the behaviour of some proteins under similar conditions, and leads to the inference that disinfection of bacteria by this means is due to hydration of their constituent proteins.

The suggestion has already been made that the difference in resistance to heat of vegetative and sporing forms is caused by the difference in the readiness with which their respective proteins are coagulated (Lewith, 1890, Hewlett, *loc. cit.* p. 20). Lewith (p. 349) adduces some experimental support for the view that this difference is to be attributed to a diminution in the water-content of spores. That coagulation is not a pure temperature effect is clear from the comparative security of both bacterial spores and proteins at high temperatures in the absence of water.

The analogy takes a more definite shape when the foregoing experiments with bacteria are compared with some recent work on the alteration of proteins by hot water.

Famulener and Madsen (1908) found that the destruction of three antigens when heated in the presence of water proceeded logarithmically and that the process had a very high temperature coefficient, being about 2 for a rise in temperature of 1° C. A similar set of results have been obtained by Madsen and Streng (1909) for a series of agglutinins.

The "heat coagulation of" two pure crystallised proteins, haemoglobin and egg-albumen, has recently been shown (Chick and Martin, 1910) to be an orderly time-process, the rate of which varies with alteration of temperature, reaction and other conditions. In the case of haemoglobin coagulation takes place logarithmically, the coagulation rate at any moment being proportional to the concentration of uncoagulated protein. In the case of crystallised egg-albumen the reaction is of a higher order, as the crystals consist of a series of salts of protein with the acid used in their preparation, which salts coagulate at different rates. The effect of temperature upon the rate of reaction was found to be unusually great, the temperature coefficient in the case of haemoglobin being 1·3 per degree centigrade, and, in the case of egg-albumen, 1·91 per degree centigrade.

These observations on proteins show that it is improper to speak of a protein as having a definite "coagulation temperature"; for the same reason, it is no longer permissible to talk of the "thermal death-point" of any particular species of bacteria. It is in both cases the possession of exceedingly high temperature coefficients which have rendered observation of these supposed constants of any practical value. This will be clear from some such example as the following. In the case of disinfection of *B. typhosus* with hot water, the temperature coefficient has been determined to be 1.635 for 1° C. rise in temperature. From the value of velocity constant in Table X, 0.111, it can be calculated that a suspension of *B. typhosus*, containing 100,000 bacteria per c.c., would be disinfected (the number reduced to less than 1, an average of 0.5, per c.c.) in 48 minutes at 49° C. From the above value of the temperature coefficient it can be calculated that the disinfection would take about 2 hours at 47°, 18 minutes at 51° C., 7 minutes at 53°, 2½ minutes at 55° and 21 seconds at 59°. If, therefore, a suspension of *B. typhosus* in water were gradually heated up, death would apparently take place suddenly at a temperature near 55° C., and it is easy to understand how some such temperature should come to be regarded as the "thermal death-point" for this species.

Another point of analogy between the "heat coagulation" of proteins and disinfection of bacteria by hot water is that both processes are similarly affected by the presence of minute quantities of acid, the rate being greatly increased in each case.

The striking similarity between the effects of temperature (dry) on the one hand, and hot water on the other indicate that disinfection by the latter is due to the action of water (coagulation or alteration) upon some one protein which is essential for the life of the bacterium, and that the character of the reaction is conditioned by the chemical action of water upon its constituent proteins.

(6) Summary of Section III.

1. (a) In the case of *B. typhosus*, *B. coli commune*, and *B. pestis* disinfection with hot water also proceeds according to a logarithmic law, the rate of disinfection at any moment being proportional to the concentration of surviving bacteria.

(b) In the case of *B. paratyphosus* the rate of disinfection at the beginning of the experiments was in excess of the theoretical, but in the course of a few minutes slowed down to a consistent rate. The same

phenomenon had previously been observed in disinfection of this species with phenol and other disinfectants.

(c) In the case of *Staphylococcus* there was the greatest variability in the character of the disinfection process, which in some cases conformed to the ideal type (anthrax spores or *B. typhosus*), in others to that of *B. paratyphosus*, while in others again there was a period of "lag" at the beginning of disinfection recalling disinfection of *Staphylococcus* with phenol. Further complications were introduced by the lack of constancy in resistance to disinfecting agents which is characteristic of this species.

2. The effect of difference in temperature upon rate of disinfection by hot water has been shown to be consistent throughout the small range investigated and in accordance with the law of Arrhenius, or some similar logarithmic law. The temperature coefficient has been determined in some cases, and in that of *B. typhosus* reaches the high figure of 1.635 per 1° C., or 136 per 10° C. In the case of *B. coli commune* and *Staphylococcus pyogenes aureus*, the temperature coefficient was not studied with the same completeness but, from the few observations made, appears to be 1.28 and 1.4 respectively per degree centigrade.

The high temperature coefficient is comparable to that obtained for precipitation of proteins with hot water, and supports the view that disinfection of bacteria by this means is due to "heat coagulation" of their constituent proteins.

3. The presence of minute quantities of acid or alkali too small to produce any direct disinfectant action has a very marked influence upon disinfection by hot water. In both cases the rate of disinfection is increased, but in the case of acid to a much greater extent. Whereas, in the case of *B. typhosus*, the addition to distilled water of sufficient alkali to render the solution $\frac{N}{7000}$ alkaline increased the mean rate of disinfection at 54° C. about 1.5- to 2-fold, a similar addition of acid increased it 5- to 7-fold. Further addition of alkali influenced the disinfection-rate comparatively little; with further addition of acid it became too rapid for study. This presents a close analogy with the influence of small quantities of acid upon "heat coagulation" of proteins.

4. The effect of reaction was of the same order when suspensions of bacteria in broth were substituted for those in water. For this reason it is impossible to obtain standard conditions in the manufacture of vaccines, as owing to the growth of the bacteria it is impossible to control the reaction of the broth.

IV. DISINFECTANT ACTION OF SUNLIGHT AND DRYING.

Sunlight. The only suitable data upon this subject, which are available, are the results of some experiments by Clark and Gage (1903) in which the disinfectant action of sunlight was quantitatively studied upon *B. typhosus* and *B. coli commune* suspended in water. Most of the experiments give irregular results from which no conclusion can be drawn. In Table XXVII, however, are set forth the results of one experiment with *B. coli* which gives consistent results and which well repays analysis. The concentration of survivors, determined from time to time

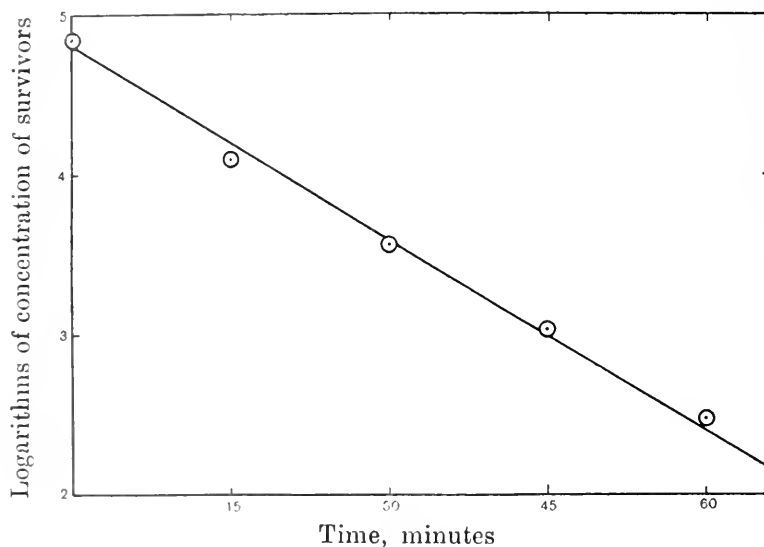


Fig. 21. Disinfection of *B. coli commune* by sunlight, results of Clark and Gage (1903), Table XXVII.

TABLE XXVII.

Disinfectant action of sunlight upon B. coli commune.

(Clark and Gage, *Thirty-fourth Annual Report, State Board of Health, Massachusetts, 1903.*)

Time, minutes = t	Concentration of surviving bacteria, numbers per c.c. = N	$\text{Log}_{10} C$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$0 = t_0$	71400 = N_0	4.854	—
15	12365	4.092	.051
30	3568	3.566	.043
45	1065	3.027	.041
60	295	2.470	.040
			Mean .044

by means of plate cultures, is seen to vary logarithmically with time, the value of the velocity constant, K , remains fairly constant in value, and a straight line is obtained when logarithms of concentration of survivors are plotted against time, see Fig. 21.

It is not justifiable to draw a definite conclusion from such scanty data, but it is more than probable, judging from analogy, that the process of disinfection by sunlight will be found to fall in line with that experimentally established for other agents.

Drying. In a preliminary examination of the suitability of the garnet¹ method for investigating the action of disinfectants upon *Staphylococcus*, Paul and Prall (1907) allowed garnets, coated with a thin dry film of this species, to remain at various temperatures and counted the surviving bacteria from time to time. At the temperature of liquid air the number of living bacteria remained roughly constant for many weeks, at ordinary temperature the number slowly decreased. Paul (1909) has recently analysed these data and shown that death—in this instance by drying—occurs in accordance with a logarithmic law and that the temperature coefficient for the process is about 2—3 for 10° C. rise in temperature (see also Paul, Birstein and Reuss, 1910).

V. SUMMARY AND GENERAL CONCLUSIONS.

1. The additional experimental evidence brought forward in the present paper lends further support to the view already put forward (1908) that disinfection is an orderly time-process, which may be considered analogous with a chemical reaction, viz. a reaction between the bacterium on the one hand, and the disinfectant on the other. In the real case disinfection proceeds in accordance with some rule analogous to the Mass Law, so that if the disinfectant is present in large excess, the disinfection rate at any moment is proportional to the concentration of the bacteria ($-\frac{dn}{dt} = Kn$, where n is the concentration of bacteria at the time t , and K is a constant depending on the temperature, concentration of disinfectant, etc.).

2. The above view was originally put forward on the basis of experiments with anthrax spores and *B. paratyphosus*, using a series of

¹ Krönig and Paul (1897).

different disinfectants (H. C. 1908). In the present paper the results of further experiments with phenol are given in confirmation, viz. with *B. typhosus*, *B. coli commune* and *Staphylococcus pyogenes aureus*.

3. Disinfection with phenol and other disinfectants has already (*loc. cit.*) been shown to be influenced by temperature in accordance with the Law of Arrhenius. The effect upon disinfection rate of alteration in concentration of disinfectant (*loc. cit.* p. 117, and H. E. Watson, 1908) is also in accord with the view expressed in 1.

4. Further confirmation has been obtained from the case of destruction of bacteria by hot water between the temperatures of 45° C. and 55° C. Investigations on this subject are described in the present paper, and a very impressive parallel shown to exist between this case of disinfection and the heat coagulation of proteins. Both are consistent time-processes proceeding in uncomplicated cases in accordance with the Mass Law, influenced consistently by change of temperature in agreement with the Law of Arrhenius, and both possessing an extraordinarily high temperature coefficient. Both are similarly influenced by addition of minute quantities of acid.

The organisms worked with were *B. typhosus*, *B. coli commune*, *B. paratyphosus*, *Staphylococcus pyogenes aureus* and *B. pestis*.

5. Disinfection by drying (Paul, 1909) and, as far as can be judged from very scanty data (Clark and Gage, 1903), by sunlight fall into line with the other cases of disinfection which have been investigated.

The facts relating to the disinfection process have not been questioned, but some amount of criticism has been aroused by the explanation which has been suggested on the following lines.

As regards disinfection (whether by "disinfectants," hot water, sunlight, drying) a culture of bacteria consists of a uniform population. The fact that the individuals do not die all at once but at a rate proportional to the concentration of the survivors at a given moment is to be attributed to temporary and rhythmical changes in resistance which, by analogy with chemical processes, may be supposed to be due to temporary energy changes of the constituent proteins.

This simple law has been found to express the relation of mortality to time for the germicidal action of chemical disinfectants and heat upon anthrax spores and very closely, in my experiments, upon *B. typhosus*, *B. coli* and *Staphylococcus* (phenol). The deviation or departure from the law, most marked in the case of *B. paratyphosus*,

has in my experience always been in one direction¹, viz. the rate of destruction falls more quickly than would be accounted for by the change in concentration of survivors alone. Here too, I believe the rate to be determined by concentration but to be complicated by the individual bacteria possessing more or less permanent differences in resistance.

Hewlett (*loc. cit.*) and Reichel (1909, p. 152) consider that the progressive nature of disinfection can be more naturally explained by supposing it to be due to differences in resistance occurring among the various bacteria². If such were the case, disinfection would certainly be gradual and not sudden, but the rate would only be proportional to the concentration of survivors if the different resistances were allotted according to one definite arrangement, an arrangement which could only occur as a remote coincidence, and could hardly be universally present. For, suppose that the disinfection of 100,000 bacteria is in question, that the survivors vary logarithmically with time, as in the ideal case of disinfection, and that for the sake of argument the rate happens to be a reduction to $\frac{1}{10}$ th in each minute,

$$10,000 = \frac{1}{10} \text{ of } 100,000, \text{ survive after 1 minute,}$$

$$1000 = \frac{1}{10} \text{ of } 10,000, \text{ will survive after 2 minutes, and}$$

$$100 = \frac{1}{10} \text{ of } 1000, \text{ will survive after 3 minutes,}$$

$$10 = \frac{1}{10} \text{ of } 100, \text{ will survive after 4 minutes, and so on.}$$

If the cause of this is to be sought in the possession by the bacteria of different individual resistances to disinfection we should have to suppose that these resistances had the following distribution among the 100,000 bacteria, viz. that

90,000 take 1 minute to kill,

9000 take 2 minutes to kill,

900 take 3 minutes to kill,

90 take 4 minutes to kill, and so on.

¹ Cases in which departure from the logarithmic law is in the direction of preliminary lag may be explained by supposing it to be due to a delay in the disinfectant getting to work; after such delay disinfection usually proceeds in the ideal way, disinfection being proportional to the concentration of survivors (see disinfection of *Staphylococcus*, p. 244).

² A similar view was expressed by Bellei (1904).

Or, in the general case, if N bacteria die logarithmically, and if $\frac{1}{x}$ of N survive 1 minute, then

$$\frac{1}{x^2} N \text{ will survive 2 minutes,}$$

$$\frac{1}{x^3} N \text{ will survive 3 minutes, and so on,}$$

and the resistance of the various individuals will be so distributed that there are present

$$N - \frac{N}{x}, \text{ or } N \frac{(x-1)}{x}, \text{ which are killed in 1 minute,}$$

$$N \left(\frac{x^2-1}{x^2} - \frac{x-1}{x} \right), \text{ or } N \frac{(x-1)}{x^2}, \text{ which take 2 minutes to kill,}$$

$$N \left(\frac{x^3-1}{x^3} - \frac{x-1}{x^2} - \frac{x-1}{x} \right), \text{ or } N \frac{(x-1)}{x^3}, \text{ which take 3 minutes to kill,}$$

and no other distribution will satisfy the necessary condition.

A further argument can be brought against a theory of variable permanent resistance, viz. that a chance characteristic is as a rule distributed in a different manner. Those individuals possessing it in moderate amount are usually in the greatest number, while those possessing it in greater or less degree are in the minority. In the case of disinfection, those apparently possessing the least resistance are invariably in the greatest number.

A more rational explanation is based upon the essential similarity of the individual bacteria, for in no other way can the logarithmic nature of disinfection in the ideal case be reasonably accounted for. If one condition be necessary for death, or a multitude of small causes, among a number of similar individuals, the result to be governed by the law of probability, death will occur logarithmically (see Yule, 1910). In order to obtain a mental picture of the disinfection process, one is led to seek an analogy with other processes, which also occur logarithmically, viz. chemical reactions of the first order such as the decomposition of hydrogen arsenide, the inversion of sugar and, under certain conditions, the "heat coagulation" of proteins. The closest possible analogy has been shown to exist between the last instance and the destruction of bacteria by hot water. In these cases an explanation has been sought in temporary changes in the energy of the molecules, as a consequence of which all molecules do not possess the same sensibility to attack at the same moment. Some such property is therefore attributed to the

molecules (or aggregates of molecules) of the constituent protein of the bacteria, whereby at a given moment only a certain proportion is liable to attack; the amount being dependent upon the concentration at the moment of unaltered protein, in other words the number of bacteria¹ surviving in unit volume.

Disinfection, whether by disinfectants or by heat, may be considered analogous to a chemical reaction the velocity of which is controlled by external conditions such as temperature or concentration of bacteria and disinfectant.

In conclusion, I gratefully acknowledge the great help I have received from Dr C. J. Martin, F.R.S., whose valuable advice and practical assistance have been at my disposal throughout this work. I am also indebted to him for the design of the very convenient apparatus, shown in Fig. 8, used for investigating disinfection by hot water.

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GUINEA-PIG EPIZOOTIC ASSOCIATED WITH AN ORGANISM OF THE FOOD-POISONING GROUP BUT PROBABLY CAUSED BY A FILTER-PASSER.

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(From the Lister Institute of Preventive Medicine.)

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*Introductory*¹.

DURING the past winter we have had an opportunity of studying two epizootics which broke out amongst the stock guinea-pigs in the Lister Institute, Chelsea. The first occurred in a room in which were nearly 200 guinea-pigs. This epidemic began in the third week of October and progressed so rapidly that by the second week of November all the animals had died. The second epidemic affected a stock of about 300 animals which were housed in a different room in the animal house, the mortality in this case being about 90 per cent. This outbreak occurred in the first week of November and lasted about five weeks. All the animals in both stocks were young, weighing on an average about 250 grammes.

In both instances the animals were allowed to run freely about the floor of the rooms. Sick animals showed a roughening of the fur, and when placed on the side were unable to regain the upright position apparently owing to a weakness of the limb muscles.

The post-mortem appearances varied a good deal. The intestines frequently showed congestion. The liver and spleen also were usually congested and occasionally contained small grey or yellow nodules. In some of the cases the suprarenals showed varying degrees of congestion, and patchy congested areas were often seen in the lungs. Effusions into the serous cavities did not occur.

Characters of the bacillus isolated.

Cultures made from the organs either after direct plating on lactose bile salt agar or after preliminary incubation in dulcitate bile salt broth gave, in a large proportion of the cases, pure growths of a bacillus which from its cultural characteristics evidently belonged to the paratyphoid group of organisms. This bacillus has been identified as being closely related to the *B. suispestifer* (the hog-cholera bacillus) and to the *B. aertryck*: indeed it would appear that these three organisms are indistinguishable by every known test including those of agglutination and absorption. The organism was isolated most easily from the contents of the small intestine. Thus it was recovered in pure culture in 12 out of 16 examinations. It was generally easy to obtain cultures from the faeces of the epizootic stock animals, at least three or four days before the death of the animals. The

¹ For the sake of brevity, extended references to the details in the Tables have been omitted in the text.

liver and spleen yielded cultures of the bacillus and less frequently it was obtained from the heart-blood. When present in the heart-blood the colonies on the plates were not numerous—a marked septicaemia did not appear to exist. On three or four occasions in which the bacillus was isolated from the faeces three or four days before death, cultures from the heart-blood and liver failed to give positive results. The bacillus was isolated from the bile once, but not at all from the urine in five cases examined.

Pathogenicity experiments.

The pathogenicity of the bacillus was tested on guinea-pigs, rats, mice and rabbits. The bacillus is extremely pathogenic to guinea-pigs when given subcutaneously but does not readily kill them when given per os. The results of pathogenicity tests are exhibited in Table I. Shortly stated these results are as follows. For guinea-pigs of 250 grms. weight doses varying from 0·0001 to 0·000001 c.c. of a young broth culture inoculated subcutaneously proved fatal in some instances only, but doses of 0·001 c.c. invariably caused death in about five days.

A dose of 2 c.c. given subcutaneously to white rats was fatal in six days, while 1 c.c. inoculated intraperitoneally killed in 24 hours.

A dose of 0·01 c.c. either intraperitoneally or subcutaneously proved fatal to mice in two days.

When using rabbits we found that 0·1 c.c. injected subcutaneously killed an animal of 900 grms. in two days, 0·1 c.c. intraperitoneally killed a rabbit of 1300 grms. in 24 hours, and 0·01 c.c. given intravenously killed in five days, the weight of the animal being 1300 grms.

Post-mortem appearances of guinea-pigs inoculated subcutaneously.

The local reaction is the principal feature post-mortem, there being in acute cases intense haemorrhagic oedema, in those dying later local necrosis or abscess. It was rare to find even minute spots in the spleen and liver, and it seems doubtful whether by subcutaneous inoculation or feeding one can produce the yellowish white nodules seen in the epizootic animals. There were no other constant features in the artificially inoculated animals.

*References to epizootics associated with bacilli of the
food-poisoning group.*

References in the literature to guinea-pig epizootics associated with this bacillus are scanty. Kovářík (1903) has given an account of an epizootic amongst guinea-pigs in Budapest which he ascribed to a variety of *B. coli*. Lehmann and Neumann, however, include the bacillus in the Gaertner group. MacConkey (1905) notes that an organism indistinguishable from *B. enteritidis* (Gaertner) seemed to be the cause of an epidemic amongst the experimental guinea-pigs at the serum department of the Lister Institute near London. One of us (G. F. P.) some years ago observed an epizootic amongst young guinea-pigs in the laboratory stock in Bombay associated with an organism giving the cultural reactions of the Gaertner group. The disease occurred in the monsoon season and affected only young animals. Eckersdorff (1908) has given a brief description of a guinea-pig epizootic in Frankfurt-a-Main associated with a bacillus of the paratyphoid group.

Filtration experiments.

There can be no doubt as to the close association of the bacillus described above with the epizootic disease. But the disease evidently presents some analogies with swine fever, a disease which has been proved to be due to a filter-passing virus,—the associated *B. suispestifer* so frequently cultivable from the organs being merely a secondary invader of no etiological importance. Dr F. A. Bainbridge carried out some filtration experiments with the organs of infected guinea-pigs in this epizootic, but was unable to pursue the investigation. The details of our experiments dealing with this point are displayed principally in the tables, but we may comment here on the experiments as a whole.

The first experiment we carried out, although not a filtrate experiment, gave results of such significance that it was considered worth while to experiment with the filtered organs of the epizootic animals. The experiment consisted firstly in sowing 0.003 c.c. of heart-blood from each of seven guinea-pigs which had died of the epizootic disease into dulcete-broth—a medium which is known to be of especial service in the detection of organisms of the paratyphoid group.

The culture tubes from three of them gave a growth of the bacillus and further results from these animals are not included in the table.

Table II. shows the results of subcutaneous inoculation into each of two guinea-pigs of minute quantities (0·0003 c.c) of the four samples of heart-blood giving negative cultures. All these animals died in periods varying from 7 to 14 days.

TABLE II.

Experiments in which minute amounts of heart-blood from infected guinea-pigs were inoculated into healthy guinea-pigs.

No. of guinea-pig	Dose of blood inoculated	Cultures from H.B. and intestines	Days lived
1	0·0003 c.c. subcutaneously	Negative	12
1 <i>a</i>	„ „	„	14
2	„ „	„	9
2 <i>a</i>	„ „	„	15
4	„ „	H.B. = negative Intestines = positive	9
4 <i>a</i>	„ „	Negative	12
6	„ „	„	7
6 <i>a</i>	„ „	„	14

In this experiment the objection may be raised that the inoculation test is usually a more delicate one than the cultural when a pathogenic bacillus is present in small numbers, and that these guinea-pigs may have died as the result of an infection by the bacillus in spite of the negative cultures. Against this view is the fact that the bacillus was isolated after death from only one of the eight animals. Moreover there was not present in any of the dead guinea-pigs the characteristic local lesion which invariably follows the inoculation of cultures of the bacillus subcutaneously.

We must regard this experiment then as strongly suggesting that some organism other than the *B. suipestifer* was responsible for the deaths of the inoculated animals.

Methods used in the filtration experiments.

The filtrates used in the experiments were obtained by first of all grinding up the organs—generally the spleen, liver, lungs and heart with sterile sand in a sterilised mortar. Sterile salt solution was added in varying amounts during this process and subsequently, the object being to secure an extract of the organs in saline of such a consistency that the filtration of the fluid through the Berkefeld filter should be fairly rapid and completed within one hour. The amount of saline

added to the organs of each guinea-pig varied from 40 to 120 c.c. The material after grinding was filtered through a Buchner's filter connected up with a vacuum pump. This filtrate was finally filtered through the sterilised Berkefeld filter. The sterility of the filtrates was tested in every instance by sowing 3 or 4 c.c. into dulcete bile salt broth and into ordinary peptone broth.

Comments on filtrate experiments.

Table III. In this series a moribund pig from the epizootic stock was killed and the organs ground up and filtered. The post-mortem appearances were typical and the bacillus was isolated from the contents of the small intestine. It will be seen that a large proportion of the guinea-pigs inoculated by various methods (subcutaneous, intraperitoneal, intracerebral, intravenous, intranasal and feeding) with the filtrate died. Special attention may perhaps be directed to the three animals fed with the filtrate and the three inoculated nasally, since these two methods, as in the case of swine-fever, are probably natural modes of infection.

From the table it will be seen that the filtrate experiments were carried on serially, the filtrates of dead animals being in their turn used for further inoculation experiments, and so on. Some indications are met with of a diminution of virulence of the infecting agent as a result of passage. A similar phenomenon has been noted by Dorset in his experiments with the filter-passer of swine fever.

In addition to the experiments with the filtrates of organs of epizootic guinea-pigs, experiments with filtered serum, bile and urine were performed and the results are recorded in Table IV.

Table V. This table summarises experiments with the filtrates of organs from several of the animals which died as a result of the inoculation of minute amounts of blood from guinea-pigs dying during the epizootic (*vide supra*).

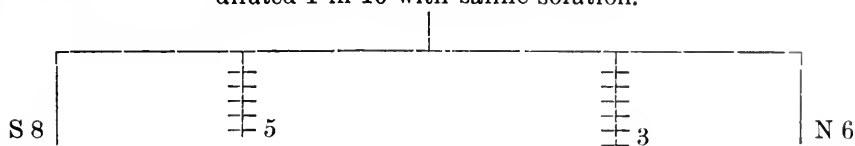
Post-mortem appearances and culture results of experimental animals.

The only fairly constant pathological feature met with post-mortem was a reddening of the wall of the small intestine.

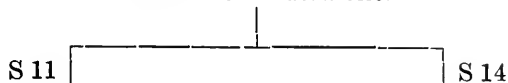
In the animals inoculated intracerebrally with 0.3 c.c. of the filtrate the brain showed extreme congestion. Marked congestion of the lungs was found in the guinea-pigs inoculated nasally with the original filtrate (see Table III.).

TABLE IV.

Filtrate of urine collected from eight dead epizootic guinea-pigs, and diluted 1 in 10 with saline solution.



Filtrate of diluted bile.



Filtrate of diluted serum from eight guinea-pigs observed to be sick and killed.

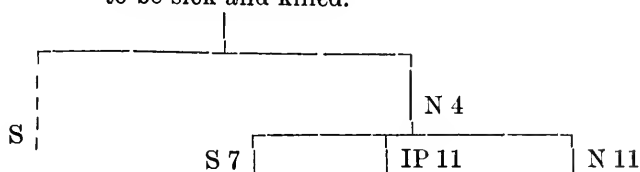
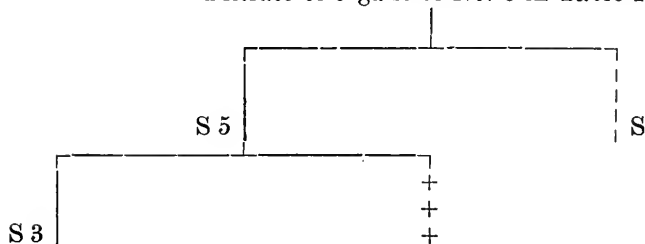
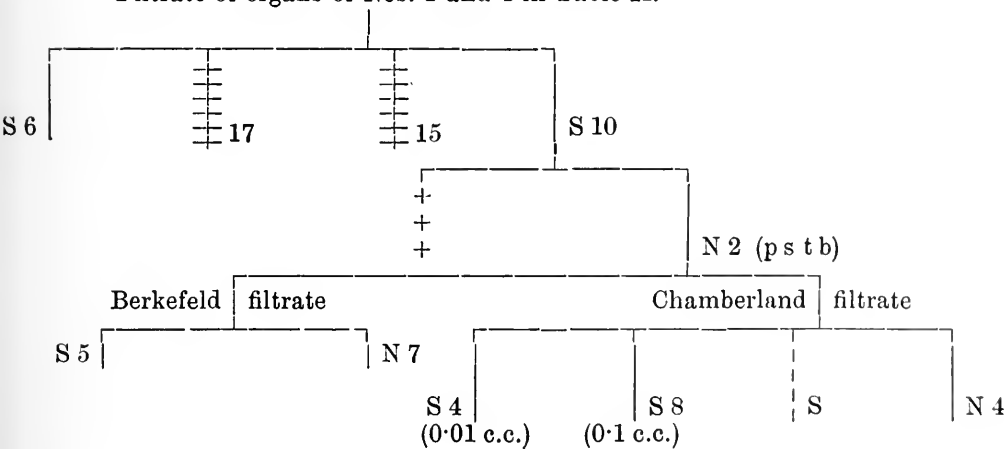


TABLE V.

Filtrate of organs of No. 6 in Table II.



Filtrate of organs of Nos. 2 and 4 in Table II.



The feeding experiments in which 60 c.c. of the filtrate were mixed with the food of three guinea-pigs may be summarised thus. Animal No. 1 (see Table III.) died in seven days, with congestion of the liver, pancreas and upper part of the small intestine. Cultures from the liver, heart-blood and small intestine gave no growth of the bacillus.

Animal No. 2 died in eight days, with congestion of the liver, small intestine and lungs; the bacillus was not found in the contents of the small intestine or in the heart-blood.

Animal No. 3 died in nine days, with congestion of the intestines, the heart-blood and intestinal contents giving no growth of the bacillus.

Cultures. In only three of 49 animals dying after the inoculation of filtrates was the bacillus recovered, although in every case the heart-blood and intestinal contents were sown into dulcete bile salt broth.

Attempts to cultivate a virus from the heart-blood and from the filtrates of organs.

The media used for growing this hypothetical virus were mixtures of agar with guinea-pig blood, guinea-pig serum, and filtrates of the organs of normal guinea-pigs. To these were added varying minute amounts of the heart-blood of recently dead guinea-pigs from the infected stock and of filtrates of the organs of infected pigs. The cultures were kept for two to three weeks at 37° C., some aerobically and some anaerobically. Two of these cultures were examined by the "dark-ground" method of illumination and showed innumerable extremely minute bodies with very active Brownian movements. The appearances exactly resembled those described by Flexner and Lewis in their culture tubes of the virus of anterior poliomyelitis. In several instances the heart-blood taken directly from dead animals was examined by this method, but showed nothing unusual nor did control uninoculated culture tubes contain any of these minute bodies.

Four of the culture tubes to which the filtrate of the organs of the animal furnishing the material for the experiments in Table III. was added were incubated for a fortnight aerobically, and from them varying amounts of fluid were drawn off and used for subcutaneous inoculation. Nine of the twelve inoculated guinea-pigs died (see Table VI.), animals 1 to 8 being inoculated from one culture tube; of these six died after periods roughly proportional to the dosage. Of the four guinea-

igs inoculated with material from the remaining tubes only one survived. These experiments are interesting, but do not of course necessarily prove any multiplication of the virus in the culture tubes.

TABLE VI.

Showing results of inoculation into guinea-pigs of contents of culture tubes containing organ-juice filtrates.

No. of guinea-pig	Dose	Culture inoculated	Result
1	0.001 c.c.	296 <i>d</i>	Survived.
2	"	"	Died in 10 days.
3	0.01 c.c.	"	Survived.
4	"	"	Died in 19 days.
5	0.1 c.c.	"	Died in 5 days.
6	"	"	" "
7	1 c.c.	"	Died in 3 days.
8	"	"	" "
9	2 c.c.	296 <i>a</i> , <i>b</i> , and <i>c</i>	Died in 10 days.
10	"	" "	Survived.
11	3 c.c.	" "	Died in 19 days.
12	"	" "	Died in 26 days.

Control experiments.

A number of control experiments was done, animals being injected with the filtrates of organs of apparently healthy stock animals and with the filtrates of organs of animals killed by subcutaneous inoculation of the bacillus. Unfortunately the whole series was vitiated by the sudden appearance of *B. pseudo-tuberculosis rodentium*. Several animals amongst the Institute stock at this time died with the post-mortem appearances of this disease and *B. pseudo-tuberculosis rodentium* was recovered from them. A considerable number of the control animals also died of this disease. Fortunately this complication did not occur until after the completion of most of the filtrate experiments.

We consider it unnecessary to burden our paper with full details of these control experiments, since we ourselves have found it impossible to draw any clear deductions from them.

We may add that several filtrates prepared from epizootic material were kept six weeks in the cold room and were then retested by subcutaneous inoculation on healthy guinea-pigs. Very few of the inoculated animals died, apparently showing that the filtrates had lost their virulence.

Discussion as to the causal agent of the disease and its transmission—Bacillus or Filter-passer?

The relation of the bacillus to the epizootic.

Before we can accept a filter-passer as the essential infective agent, it must be shown that the associated bacillus is in itself incapable of spreading the disease. Now if we assume that this bacillus is the causal agent of the disease, it must be supposed that the natural mode of infection is either subcutaneous or per os.

I. Possible entry of the bacillus through the skin.

The bacillus might be inoculated by lice, for guinea-pigs are infested with lice and with no other parasites; but against transmission by lice is the fact that the septicaemia when it did occur in naturally infected animals did not appear to be a marked one, so much so that in a considerable proportion of cases the bacillus could not be cultivated from the blood. Thus, out of cultures made from the heart-blood of fourteen epizootic guinea-pigs only three were positive.

The following experiments bearing on the question of transmission by lice were carried out:

1. A healthy stock guinea-pig was tied down in a tray and the corpse of a guinea-pig which died during the epizootic and which was covered with lice was left in contact with it for five minutes. The lice certainly transferred themselves, for they were seen attached to the ends of the hairs of the stock animal after the corpse was removed, a position in which they are never seen under ordinary circumstances in the case of a living guinea-pig. The stock guinea-pig was kept under observation for three or four months but showed no symptoms indicating an infection.

2. Some lice from the fur of a guinea-pig dying two days after the subcutaneous inoculation of one c.c. of a broth culture of a strain of the bacillus recovered during the epizootic were transferred directly to a healthy guinea-pig. The animal survived.

It may be added that no appearance was found in the animals dead of the disease which suggested that the bacillus gained entry through the skin. In animals artificially inoculated subcutaneously there is found an intense haemorrhagic oedematous infiltration at the site of injection.

II. *Possible transmission of the bacillus by feeding.*

Attempts were made to infect animals by feeding them with broth cultures of the bacillus, in some cases a single contaminated meal being given, in others several doses of culture and in one series massive doses were repeatedly given with food (Table VII.). On the whole these experiments confirm the observations of various workers, that feeding guinea-pigs with bacilli of the paratyphoid group is an uncertain method of infection.

The bacillus was recovered from about one-third of the pellets of faeces picked from the floor of the room in which the infected stock pigs were confined, so that it may be supposed that opportunities for the contamination of food with the bacillus were numerous, but the uncertainty of feeding as a mode of infection above referred to considerably lessens the significance of these observations in relation to the natural spread of the disease.

The bacillus was not recovered from the dust collected from crevices a short distance above the floor.

III. *Possible transmission of the bacillus by contact with infected animals.*

Healthy guinea-pigs were put into the same cages with animals inoculated subcutaneously with varying doses of the bacillus and with others fed with the bacillus, but few of them died and from them the bacillus was rarely recovered (Tables VII and VIII.).

In this connection it is worth remembering that the intestinal contents of animals subcutaneously inoculated with fatal doses of the bacillus almost invariably contained the bacillus, and often in pure culture.

TABLE VII.

Showing the results of feeding experiments and of putting healthy guinea-pigs as contacts with the fed animals.

No. fed	Dose of cultures	Result	Cultures from fed guinea-pigs	No. of contacts	Result	Cultures from contact guinea-pigs
3	12 c.c. 4 days broth culture each	All survived	—	6	All survived	—
4	One 3 days agar slope each	Killed 49 days later	Faeces of all 9 days later positive. Small intestines and organs when killed all negative	8	All survived	—
1	6 c.c. 24 hrs. broth cult. on 3 occasions	Survived	Faeces + 5 wks. after last feeding	2	Both survived	—
1	"	"	Faeces + 22 dys. after last feeding	2	Both survived	—
1	"	"	Faeces + 5 wks. after last feeding	2	1 died, 1 survived	H. B., Liver, Intestine, all negative.
1	"	"	Faeces + 3 wks. after last feeding	2	1 died, 1 survived	H. B., Intestine, negative; Spleen positive.
1	"	"	Bacillus not recovered in faeces	2	1 died, 1 survived	H. B., Liver, Intestine, all negative.
1	"	Died in 4 days	Liver and intestine +	—	—	—
2	6 c.c. 48 hrs. broth culture each	Both survived	—	4	2 survived, 2 died	— Cultures negative.
1	"	Died	Liver and intestine positive	2	2 survived	—
11	60 c.c. 24 hrs. broth culture	All survived	—	0	—	—
4	"	Died in 13 days	H. B. of all positive	0	—	—
1	"	Died in 5 days	H. B. = 0	0	—	—
1	"	Died in 6 days	—	0	—	—
1	"	Died in 7 days	H. B. = 0	0	—	—
1	"	Died in 14 days	H. B. = +	0	—	—
1	"	Died in 15 days	H. B. and intestines = 0	0	—	—

TABLE VIII.

Showing results of subcutaneous inoculation of the bacillus into guinea-pigs together with the results of contact experiments with the inoculated guinea-pigs.

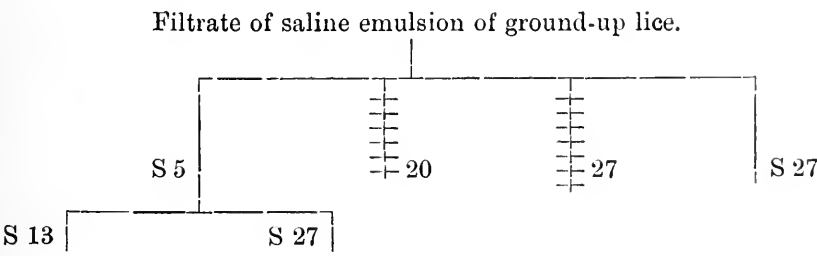
No. of guinea-pig	Dose	Days lived	Culture from small intestine	No. of contacts used	No. of contacts surviving
1	0.1 c.c.	4	Bacillus recovered	2	2
2	„	2	—	2	2
3	0.01 c.c.	4	Bacillus recovered	2	2
4	„	3	„	2	1
5	„	7	„	2	2
6	0.001 c.c.	7	„	2	2
7	„	7	„	2	2
8	„	5	„	2	2

THE RELATION OF THE FILTER-PASSER TO THE EPIZOOTIC.

I. Possible entry of the virus through the skin.

The bodies of two guinea-pigs dying during the epizootic were placed in the hot room for two hours, and the lice which soon came out to the extreme ends of the hairs were picked off, ground up with normal saline solution in a mortar and the emulsion filtered through a Berkefeld filter. The ground-up lice before filtering gave no growth of the bacillus in dulcitate broth and the filtrate proved sterile on inoculation into broth. Animals were inoculated with the filtrate, the results being set forth in Table IX.

TABLE IX.



II. Possible entry of the virus by feeding or nasal infection.

Some experiments dealing with this point were carried out and have been already referred to.

III. "*Contact*" experiments.

It is a matter for some regret that especially during the earlier experiments when we had at our disposal a healthy stock of animals for experimental purposes contact experiments were not carried out. At this period the supply of guinea-pigs was somewhat restricted, and we preferred to use them for filtration experiments in order to discover whether or not the filtrates were pathogenic. We fully realise the importance of contact experiments in an investigation of this kind and should another opportunity arise in future we would endeavour to settle this point.

OCCURRENCE OF THE BACILLUS IN GUINEA-PIGS APART FROM AN EPIZOOTIC.

The bacillus was found in a high percentage of cases (*vide supra*) in the faeces and intestinal contents of guinea-pigs from the stocks affected by the epizootic between the second week in October and the first week in December. During this year the very large number of guinea-pigs under observation in connection with the routine examination of milk for tubercle bacilli have given the bacillus in only five instances in the heart-blood and organs, the intestinal contents not being examined as a rule. In May, two guinea-pigs out of four dying as the result of the injection with milk contained the bacillus in their intestines.

Six apparently healthy guinea-pigs were obtained in December from the totally unrelated stock at Elstree, where at the time no epizootic existed. Three of them were killed, and the organs were found to be quite healthy and gave no cultures. Of the other three one showed two small hard whitish yellow nodules in the spleen, which, however, gave no culture of the bacillus. The second showed a few white necrotic patches in the liver and four or five small whitish nodules in the spleen; a bacillus indistinguishable from that found in the present epizootic was cultivated from the spleen but not from the intestinal contents. The third showed a few yellow-white areas in the liver, and three or four yellowish white nodules in the spleen which gave a culture of the same bacillus.

At this time a few more deaths than usual were noted amongst the Elstree breeding-does but no definite epizootic existed. Dr MacConkey

kindly forwarded us two of these does ; both of them showed numerous nodules in the liver and spleen and gave pure cultures of the bacillus.

It is of interest to note that similar appearances to these suggesting chronic infection were frequently observed in guinea-pigs from the stock affected by the epizootic which died at various periods of some four to eight weeks after the height of the epizootic, and from many of these the bacillus was obtained in pure culture.

In December, the faeces of 15 guinea-pigs from a separate stock which remained unaffected by the epizootic were examined for the presence of the bacillus with negative results. Guinea-pigs from this stock were used for the earlier filtrate experiments, and as stated above gave cultures of the bacillus in 6 % of cases.

Between November and May the intestinal contents of 63 guinea-pigs used for inoculations in the ordinary routine examination of milk for tubercle bacilli were examined and the bacillus recovered in five instances, *i.e.* 8 %.

We may compare this with Uhlenhuth's finding of *Bacillus suispestifer* in the intestinal contents of 8 % of normal swine.

In nearly every case the strains of the bacillus referred to in this section were tested by agglutination as well as by cultural methods (see Table X.). It will be seen that all these strains corresponded in their agglutination reactions with the epizootic strain.

TABLE X.

Showing agglutination limits of various strains of the bacillus isolated from guinea-pigs apart from the epizootic. The serum used was obtained from a rabbit immunised with a strain of the bacillus isolated during the epizootic and agglutinated the homologous organism up to 1—10000.

Wright's method used—two hours at 37°, overnight at room temp.

The final reading is given.

	G.-pig inoculated with milk deposit. Killed 28 days later, T.B. found. Culture from small intestine	G.-pig inoculated with milk deposit. Killed 28 days later, T.B. not found. Culture from small intestine	G.-pig dead as result of inoculation of milk deposit. Culture from small intestine	G.-pig dead as result of inoculation of milk deposit. Culture from small intestine	Culture from spleen of apparently healthy Elstree g.-pig	Culture from spleen of apparently healthy Elstree g.-pig	Culture from spleen of Elstree breeding-doe	Culture from spleen of Elstree breeding-doe
100	+	+	+	+	+	+	+	+
1000	+	+	+	+	+	+	+	+
5000	+	+	+	+	+	+	+	+
10000	tr	-	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-

DISCUSSION OF RESULTS.

The frequent finding of the bacillus in guinea-pigs dying during the epizootic as contrasted with its much less frequent occurrence in other guinea-pigs, at first sight strongly suggests that this bacillus was the causal agent of the epizootic.

Our results do not support this view in the following particulars:

1. It cannot be doubted that there was a spread of the bacillus from animal to animal during the epizootic as our culture results show, but the difficulty of killing guinea-pigs by feeding them with cultures of the bacillus almost negatives the idea that the epizootic was thus maintained and spread, and practically makes it necessary to postulate the presence of some other factor as the effective agent in the transmission of the disease. The migration of the bacilli from the intestines to the organs is probably merely a secondary invasion as in the analogous case of the *B. suipestifer* in swine fever.

2. This first objection is further strengthened by the results of our contact experiments in which contacts put with guinea-pigs infected either by feeding or subcutaneously and excreting the bacilli in the faeces rarely died, while the mortality in the epizootic was about 90 %.

3. Skin infection is the only other likely mode of transmission, and we have above given the evidence against this possibility.

4. We have evidence that the bacillus is found in guinea-pigs apart from an epizootic and in apparently healthy animals.

It is true, as is shown by one of us, that some of the survivors of the epizootic proved to be chronic carriers of the bacillus, that the survivors' serum definitely agglutinated the bacillus, and that they were immune to large doses of virulent cultures given subcutaneously. It must be noted, however, that in the course of a series of feeding experiments now in progress, we have found that guinea-pigs fed with cultures may excrete the bacillus in the faeces for some time subsequently while remaining apparently healthy and that the blood of some of these animals agglutinates the bacillus.

The general trend of the whole of these experiments leads us to the view that the bacillus is merely a secondary invader, and that alone it cannot explain the phenomena connected with the epizootic. We suggest that the primary factor in the transmission of the epizootic was a filter-passer and the high mortality amongst animals inoculated with sterile filtrates supports this view.

SUMMARY.

1. An epizootic killing 90 % of a stock of 500 guinea-pigs has been described; cultures from these guinea-pigs frequently gave an organism indistinguishable by cultural or serological tests from the *B. aertryck* and the *B. suispestifer*.
2. This organism was highly pathogenic when inoculated subcutaneously into guinea-pigs and of low pathogenicity when given to them with food.
3. Healthy contacts put with animals infected subcutaneously or fed with the bacillus did not die.
4. Sterile filtrates of organs of guinea-pigs of the infected stock administered by different methods frequently killed.
5. The evidence definitely suggests that the essential infecting agent in the epizootic was a filter-passer.

REFERENCES.

- ECKERSDORFF (1908). Kasuistische Beiträge zum Vorkommen von Bacillen der Paratyphus (Hog-Cholera)—Gruppe. *Arb. a. d. Königl. Inst. f. exper. Therap. zu Frankfurt-a-M.* Heft 4, p. 63.
- KOVÁRZIK, K. (1903). Meerschweinchenepizootie durch eine Varietät des Coli-bacillus verursacht. *Centralbl. f. Bakteriol., Orig.* xxxiii. p. 143.
- MACCONKEY, A. (1905). Lactose-fermenting bacteria in faeces. *Journ. of Hygiene*, Vol. v. p. 333.

PUBLICATIONS RECEIVED.

BOOKS.

BACELLAR, RODRIGUES, DUPRAT, ALVES and DA SILVA (1909). *A proposito da peste bubonica no Rio Grande*. (Memoria apresentada na sessão extraordinaria do Centro Medico di Pelotas de 6. XI. 1907.) 240 pp., 1 map. Rio Grande do Sul, Brazil: Pintos & Ca.

A study of plague in Rio Grande beginning with the year 1902. Plague in man coincides with rat-plague; it occurs in autumn. Almost all cases occurred in women, children and servants—those who are most confined to houses. Of domestic animals the cat found most susceptible to plague. Observations on epidemiology and suggestions for plague prevention.

FLEXNER, A. (1910). *Medical Education in the United States and Canada*. A Report to the Carnegie Foundation for the Advancement of Teaching, with an Introduction by Henry C. Pritchett, President of the Foundation. Bulletin iv. pp. xvii + 346. 25 × 19 cm. New York City: 576 Fifth Avenue; Boston: The Merrymount Press, D. B. Updike.

This report on medical education forms the first of a series of reports on professional schools to be issued by the Carnegie Foundation. It deals with some 150 schools including what is good and bad (homeopathic, eclectic, osteopathic and "what not") with the object of gathering together information with which the public in general is as much concerned as the regular profession. The Report is divided into two parts dealing (1) with the history of medical education in America; the present status of medical education is fully described and "a forecast of possible progress in the future is attempted"; (2) gives a detailed description of the existing medical schools in each State of the United States and in each province of Canada. A similar study of medical education in Great Britain, Germany and France is about to be proceeded with.

"The striking and significant facts which are here brought out are of enormous consequence not only to the medical practitioner, but to every citizen of the United States and Canada; for it is a singular fact that the organization of medical education in this country has hitherto been such as not only to commercialize the process of education itself, but also to obscure in the minds of the public any discrimination between the well-trained physician and the

physician who has had no adequate training whatsoever."—"For twenty-five years past there has been an enormous over-production of uneducated and ill-trained medical practitioners."—The report is unhesitating and just in its strictures upon the many inferior institutions in the country—it is to be hoped that it may exert a salutary effect. Such criticism as it offers can but benefit the few medical schools which have persisted in maintaining a high standard in the face of self-advertising, commercial and quack "colleges" which are strewn broadcast over the land. For those who are interested in Medical Education in any country the Report will prove highly interesting reading.

GONÇALVES, J. (1910). *Defeza Sanitaria da Europa contra a Peste*. Hygiene e Prophylaxia Internacional. 183 pp. 26 × 17 cm., paper. Lisbon: Antiga Casa Bertrand, José Bastos & Ca., 73 Rua Garrett.

This treatise is divided into three parts: I. The biological trio—rat—flea—man; II. The geographical distribution of plague at the present day; III. Plague Prophylaxis. The first part (112 pp.) deals with rat plague; plague and fleas; plague in man; epizootic, epidemic and pandemic plague. The author gives a good summary of our present knowledge regarding the etiology of plague and its prevention. He has drawn his information freely from various sources including the Reports of the Advisory Committee which have appeared in this *Journal*. The work will be very useful in spreading the knowledge which has been acquired to readers of Portuguese in many parts of the world.

HÜBENER, E. (1910). *Fleischvergiftungen und Paratyphusinfektionen. Ihre Entstehung und Verhütung*. 204 pp., 3 plates, 2 figs. and 10 curves in the text. 26 × 17 cm. Price 8 marks, paper cover. Jena: Gustav Fischer.

An important monograph which brings together what is at present known regarding the cause and prevention of meat-poisoning and infection due to *Bacillus paratyphosus*. The book is divided into ten chapters dealing with—1. The history of the subject.—2. The average annual amount of meat consumed and condemned in Germany.—3. The frequency of meat-poisoning cases.—4. Meat-poisonings due to specific bacteria of the Paratyphoid and Gärtner groups. (pp. 20—120: considered in great detail).—5. Are other bacteria concerned in specific meat-poisoning in Man and Animals?—6. Meat intoxications due to non-specific bacteria.—7. Botulism.—8. Prophylaxis of meat-poisoning.—9. Medico-legal aspect of meat-poisoning.—10. Paratyphoid infections which are not due to meat consumption. Bibliography (pp. 190—204: very complete). The work can be thoroughly recommended.

KELYNACK, T. N. [Editor] (1910). *Infancy*. National Health Manuals. 186 pp. 19 × 12 cm. London: Methodist Publishing House, 25 City Road, E.C. Price (paper covers) 1s. net.

The first of a series of manuals intended "to afford concise and up-to-date scientific presentation of the principles and practices which guide and govern the establishment and maintenance of personal, domestic, and national health" for the use of laymen. The little book contains twelve chapters including: I. An Introduction by the Editor.—II. The Anatomy and Physiology of the Infant, by J. B. Hellier.—III. The Hygiene of Infancy, by Sir Wm. J. Thompson.—IV. The Feeding of Infants, by J. S. Fowler.—V. Common Disorders of

Infancy and their Prevention, by A. Dingwall-Fordyce.—VI. Schools for Mothers, by D. E. L. Bunting.—VII. The Rôle of the Crèche or Day Nursery, by F. S. Toogood.—VIII. Milk Dépôts and Kindred Institutions, by J. J. Buchan.—IX. Law and Infant Life, by S. B. Atkinson.—X. The Infant and the Nation, by Sir J. W. Byers.—XI. Municipal Action in the prevention of Infantile Mortality, by J. T. J. Sykes.—XII. Moral aspects of Infant Life Protection, by T. Arthur Helme. All the Authors are members of the medical profession and well qualified to write on the subjects they present in their short essays.

KELYNACK, *T. N. [Editor] (1910). *Medical Examination of Schools and Scholars*, with an Introduction by Sir Lauder Brunton, Bart., F.R.S., etc., 434 pp., 22 × 14 cm. Cloth. Price 10s. 6d. net. London: P. S. King and Son, Orchard House, Westminster, S.W.

The aim of this book is to provide a guide for school medical officers which may likewise be useful to others interested in the health of school children. The volume includes chapters by no less than thirty-six contributors; useful bibliographies will be found at the end of each chapter. The book should prove very useful since it brings together a great deal of information from varied sources at home and abroad. It contains 32 essays, written by recognized authorities, as follows: Co-relation of the School Medical Service and the Public Health Service, by E. W. Hope; Organization and Administration of the Medical Examination of Schools, by G. Reid and J. Priestley; Organization and Administration of the Medical Examination of Scholars, by W. J. Howarth; Medical Examination of Boys in Preparatory and Public Secondary Schools, by C. Dukes; Medical Examination of Girls in Secondary Schools, by A. M. Corthorn; Medical Examination of Children under the Poor Law and in Orphanages and Industrial Schools, by A. D. Edwards; Medical Examination of Schools and Scholars in the British Army, by C. H. Melville; The General Routine Medical Examination of School Children, by C. Riviere; Medical Supervision of Games, Sports and Exercises, by A. I. Simey; The Eyes and Eyesight of School Children, by G. Foggin; The Ears, Nose and Throat in School Children, by A. H. Cheatele; Dental Conditions in Elementary School Children, by G. Cunningham; Mentally defective Children, by A. F. Tredgold; School Clinics, by L. Williams; The Feeding of the School Child, by J. Lambert; Open Air Schools, by R. P. Williams; The School Nurse, by D. Forbes; The Medical Examination of School Teachers, by R. T. Williams. Chapters XIX.—XXXII. deal with the Medical Examination of Schools and Scholars in Scotland (by W. L. Mackenzie), Ireland (by A. J. Lindsay), Wales (W. Ll. Edwards), Canada (H. MacMurchy), Australia (Sir P. S. Jones), New Zealand (J. M. Mason), United States (L. H. Gulick and L. P. Ayres), Germany (M. Fürst), France (L. Dufestel), Norway (Yngvar Ustvedt), Sweden (C. Sundell and G. Törnell), Denmark (P. Hertz), Switzerland (R. Schwab), and with Physical Education in American Universities, by R. T. McKenzie.

MACWEN, HUGH A. (1909). *Food Inspection, a Practical Handbook*. 256 pp., with numerous illustrations. 22 × 16 cm. Cloth. London: Blackie & Son, Ltd., 50 Old Bailey, E.C.

The book is written with the object of "giving a clear and concise account

of the inspection of meat and other foods, and the principles underlying the hygienic production of prepared foods." The author has familiarized himself with the methods employed in the chief cities of Great Britain, Germany, and the United States. The book is divided into four sections: I. The Inspection of Meat, and the Diseases commonly met with in the Abattoir.—II. The Construction and Management of Slaughter-Houses and Public Abattoirs, and the Law relating to Slaughter-Houses and Markets.—III. The Inspection of Fish, Poultry, Game, Vegetables, Fruit, etc., considered from a Hygienic Standpoint, and the Law relating to Unsound Foods.—IV. Preservation and Storage of Meat and other Foods and the Causes of Unwholesomeness in Food. The book promises to be very useful, the author having brought together much valuable information. The illustrations are good and for the most part original.

PROUT, W. T. (1909 received 1910). *Lessons on Elementary Hygiene and Sanitation with special reference to the Tropics*. 2nd ed., 159 pp., 60 text figures. 22 × 14 cm. Cloth. Price 2s. 6d. London: J. & A. Churchill, 7 Great Marlborough Street.

An elementary text-book intended for the use of schools in the tropics and written in lecture form. There are a good many people besides school-children in the tropics who might derive benefit from some of the lessons contained in the volume.

STICKER, G. (1910). *Abhandlungen aus der Seuchengeschichte und Seuchenlehre*. Bd. I. *Die Pest*. Teil 2. Die Pest als Seuche und als Plage. 542 pp., 5 text figures. 26 × 19 cm. Price, unbound, 30 marks. Giessen: Alfred Töpelmann (vormals J. Ricker).

Whereas the first part of the volume dealt with the history of plague, this second part deals with: The bacteriology of plague, its endemic centres, the spread of plague geographically, the carriers and sufferers from plague in the animal kingdom—the origin and course of plague epidemics in man—the effects and consequences of epidemic plague—preventive measures present and past. The bibliography occupies no less than 47 pages. The book shows evidence of great industry on the part of the author in collecting together historical and modern information on plague. As a work of reference the book will surely prove invaluable.

BROCHURES.

A Code of Rules for the prevention of infectious and contagious diseases in Schools, issued by the Medical Officers of Schools Association. 6th ed. London: J. & A. Churchill, 64 pp., price 1s. net.

ACORN, J. W. (1910). *Nature's Help to Happiness*. 55 pp. 18 × 12 cm. Cloth, price 1s. net. London: William Rider & Son, Ltd., 164 Aldersgate St., E.C.

KAUP, I. (1910). *Betrachtungen über die Bekämpfung der Tuberkulose in einigen Ländern, namentlich in England, Frankreich, den Vereinigten Staaten, Norwegen, Schweden und Dänemark und ihre Nutzanwendung für Deutschland*. 99 pp., 22 figs. Price 1 mark. Paper covers. 23 × 15 cm. Berlin: Carl Heymann.

A reprint from the *Zeitschr. der Zentralstelle "Concordia,"* Nos. 1 and 2, Jan.-Feb., 1910, issued in brochure form. The brochure, as explained in the title, relates to the campaign conducted against tuberculosis in various countries, and it contains a great deal of valuable information condensed into a relatively small number of pages. The illustrations relate to the various types of sanatoria which have been evolved in different countries and curves illustrate the prevalence of tuberculosis in the several countries during recent decades. The brochure offers a serious study of the subject and is well worth reading.

ROBERTSON, W. (1910). *Public Health* (Catechism Series). Part I. Water.—Part II. Air and Ventilation, Warming, Lighting and Climate.—Part III. Sewage and its Treatment.—Part IV. Vital statistics, Dwellings and Meteorology.—Part V. Epidemiology, Food, Burial, Water-closets, Disinfectants, Heating, Hospitals. Second Edition. Each part, in paper, 19 × 13 cm., consisting of about 50 pp., price per part 1s. net, or 5 parts, bound in cloth, 4s. 6d. Edinburgh: E. and S. Livingstone, 15 Teviot Place.

The second edition of this series is revised by Dr W. Robertson, Medical Officer of Health, Leith. The method of question and answer adopted in the "Catechisms" is intended to aid students preparing for Examinations in Medicine and for the Diploma in Public Health.

SCHOFIELD, A. T. (1910). *How to keep Fit.* An unconventional Manual. 79 pp. 18 × 12 cm. Cloth. Price 1s. net. London: William Rider & Son, Ltd., 164 Aldersgate St., E.C.

SCHOFIELD, A. T. (1910). *Nervousness.* A Brief and Popular Review of the Moral Treatment of Disordered Nerves. 88 pp. 18 × 12 cm. Cloth. Price 1s. net. London: William Rider & Son, Ltd., 164 Aldersgate St., E.C.

THOMSON, H. H. (1910). *Consumption, its Prevention and Home Treatment.* A Guide for the Use of Patients. (Oxford Medical Publications.) 75 pp. 19 × 13 cm. Cloth. Price 2s. net. London: Henry Frowde, Oxford University Press, and Hodder & Stoughton, Warwick Square, E.C.

The author, who is medical superintendent of the Liverpool Sanatorium, has written this little book for the guidance of consumptives and their friends, the text being based on lectures delivered by him to patients in the belief that "in the campaign against tuberculosis the educational influence which emanates from the sanatorium occupies a prominent position." The book should prove of use to those for whom it is intended.

WINSLOW, L. FORBES (1910). *The Suggestive Power of Hypnotism.* 99 pp. 18 × 12 cm. Paper. Price 1s. London: Rebman, Ltd., 129 Shaftesbury Avenue, W.C.

A little treatise written in a popular vein, and judging from its cover, likely to catch the eye of a passing traveller at a station bookstall; this class of reader will perhaps find the large type an additional inducement to peruse the booklet. It does not contain anything new but it is entertaining.

REPORTS.

Annual Report of the Medical Officers of Health and of the Chief Port Sanitary Inspector for the year 1909, including a Report on Canal Boat Inspection. Bristol Port Sanitary District. Printed by order of the Port Sanitary Committee. (1910), 44 pp. Bristol: Bennett Brothers, Ltd., Printers, Counterslip.

DELÉPINE, A. S. (1909). *Report to the Sewer Ventilation Committee upon the Effects on Health of the Air of the High Street Sewer.* 32 pp., with map and figures. Manchester: H. Blacklock & Co., Ltd.

Eighth International Tuberculosis Conference, Stockholm, 8-10 July, 1909. 542 pp. 23 × 15 cm. Issued by order of the International Anti-Tuberculosis Association under the editorship of the Secretary-General, Prof. Pannwitz. Berlin-Charlottenburg, 1910.

Contains a complete report of the proceedings of the Conference.

Fifth Annual Report of the Henry Phipps Institute for the Study, Treatment and Prevention of Tuberculosis, 1907 to 1908 (issued 1909) edited by Dr Joseph Walsh, published by the Henry Phipps Institute, 238 Pine St., Philadelphia; 463 pp. with several plates. 26 × 17 cm.

Contains contributions by members of the staff of the Institute as follows: Clinical and Sociological Report for the Year, by L. F. Flick; Studies of the Bone-Marrow in Pulmonary Tuberculosis, by W. W. Cadbury; Elimination of Tubercle Bacilli by the Intestines, by J. McFarland and E. J. G. Beardsley; Tenderness in Pulmonary Tuberculosis, especially Percussion Tenderness, by C. M. Montgomery; The Opsonic Index in Pulmonary Tuberculosis, by J. D. Blackwood; Laryngological Report for the Year, by G. M. Coates; Neurological Report for the Year, by D. J. McCarthy and H. Carncross; Fibrosis of the Lungs, by H. R. M. Landis; Pneumothorax in Pulmonary Tuberculosis, by W. B. Stanton; Comparison of the Pathological Findings etc. in 11 Cases of Tuberculosis of the Lungs, by J. Walsh; Pathological Report of the Year, by C. Y. White; Bacteriological Report of the Year, by E. Burvill-Holmes; The Thoracic Duct in Chronic Pulmonary Tuberculosis, by J. T. Ullom; Report of a Case of Prenatal Poliomyelitis, by D. J. McCarthy. As with the previous Reports, the form in which it is issued leaves nothing to be desired.

Leprosy in New South Wales. 18th Report of the Board of Health, New South Wales, for the year 1908. (1909). Sydney: W. A. Gullick, Govt. Printer. 42 pp., 1 pl., 1 chart.

Medical Report of the Montreal Maternity, prepared under the direction of the Physician Accoucheur for the Medical Board 1908 (received 1910). 29 pp.

PORTER, C. (1910). [Municipal Council of Johannesburg.] *Report of the Medical Officer of Health on the Public Health and Sanitary Circumstances of Johannesburg during the Triennium 1st July, 1906-30th June, 1909.* To which is appended a Report by the Medical Attendant (Dr P. G. Stock) on the Health of the Natives and Indians employed by the Council. 68 pp., with numerous charts and tables. 33 × 21 cm. Johannesburg: E. H. Adlington & Co.

REPRINTS.

- COUGHLIN, R. E. (2. iv. 1910). The Athletic Life in its Relation to degenerative changes in the Cardiovascular System. *Med. Record*. Reprint, 18 pp.
- DELÉPINE, S. (xi. 1909). The Manchester Milk Supply from a Public Health Point of View. *Manchester Statistical Society*. 50 pp., with maps and charts.
- DELÉPINE, S. (1910). Report on investigations in the Public Health Laboratory of the University of Manchester upon the prevalence and sources of tubercle bacilli in cow's milk. Extract from the *Annual Report of the Medical Officer of the Local Gov't. Board for 1908-9*. pp. 341-414 with maps and diagrams.
- DELÉPINE, S. (v. 1910). Contribution to the study of the influences determining the prevalence of bovine tuberculous mastitis. *Proc. Royal Soc. of Med.* 40 pp.
- FRASER, H. and STANTON, A. T. (1909, received iv. 1910). The Etiology of Beri-Beri. *Studies from the Institute for Medical Research, Federated Malay States*. Kuala Lumpur: Gov't. Printing Office.
- GAVINO, A. and GIRARD, J. (1910). El tifo experimental en los monos inferiores. —Sobre ciertos cuerpos encontrados en la sangre de los individuos atacados de tifo (Tubardillo).—El tifo exantemático en los monos inferiores (*Ateles vellerosus*). Inmunidad conferida por un primer ataque; Resistencia del virus á la calefacción. *Publicaciones del Instituto Bacteriológico Nacional*. 32 pp., 1 pl. and charts. Mexico: Tipografía de la Oficina Impresora de Estampillas, Palacio Nacional.

Reprint of two preliminary notes and a "second-communication" dealing with experiments with typhus fever conducted on monkeys: *Ateles vellerosus*. Blood (3-5 c.c.) from human cases up to the 10th day of sickness produced the disease in monkeys after an incubation period of 11-14 days. Infective blood was still virulent after heating to 50° C. for 40 minutes. Rats, mice, rabbits, dogs, pigs and horses found to be resistant.

- JAWORSKI, W. (1909). Politik und Nationalität auf den internationalen medizinischen Kongressen. *Wien klin. Wochenschr.* xxii. Reprint, 5 pp.
- JORDAN, E. O. and HARRIS, N. MACL. (1909). Milk-sickness. *Journ. Infect. Dis.* vi., pp. 401-491.
- STEPHENSON, S. and HERRINGHAM, W. P. (iii. 1910). On puerperal Amaurosis. *The Ophthalmoscope*. Reprint, 20 pp.
- Zur historischen Biologie der Krankheitserreger. Materialien, Studien und Abhandlungen* gemeinsam mit V. Fossel, T. v. Györy und W. His von K. Sudhoff u. G. Sticker herausgegeben. Heft 1, 12 pp., price 0.40 marks; Heft 2, 44 pp., price 1.40 marks. Giessen: Alfred Töpelmann (vormals J. Ricker).

The first fasciculus contains two papers: "Historik und Seuchenforschung," by K. Sudhoff and "Parasitologie und Loimologie," by G. Sticker. The second fasciculus contains a paper entitled "Die Bedeutung der Geschichte der Epidemien für die heutige Epidemiologie. Ein Beitrag zur Beurteilung des Reichsseuchengesetzes," by G. Sticker.

REPORTS ON PLAGUE INVESTIGATIONS
IN INDIA.

ISSUED BY

THE ADVISORY COMMITTEE

APPOINTED BY THE SECRETARY OF STATE FOR INDIA,
THE ROYAL SOCIETY AND THE LISTER INSTITUTE.*(Continued from Volume VIII, p. 308.)*

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XXXIII. EXPERIMENTAL PRODUCTION OF PLAGUE EPIDEMICS AMONG ANIMALS.

(Third communication.)

IN previous reports (vol. VI. p. 470 and vol. VII. p. 421), we described several series of experiments the results of which went to show that fleas and fleas alone were the transmitting agents of the infection of plague. It will be remembered that these experiments were carried out in a series of small godowns, the construction of which has been described in detail. The animals employed in the majority of previous experiments were guinea-pigs, though a limited number of experiments were carried out with monkeys. Gottschlich (Kolle and Wassermann's *Handbuch der Pathogenen Mikroorganismen*, Suppl. vol. II., 1907, p. 52) considers that the results obtained with guinea-pigs cannot be applied to rats as the latter animals, unlike guinea-pigs, feed on the carcasses of their dead companions. Gottschlich while admitting that fleas may transmit the disease from rat to rat considers this method of spread to be of only subsidiary importance. He is of opinion that, among rats, plague is chiefly spread by the healthy animals feeding on the carcasses of the plague infected. In order to examine the validity of these criticisms we decided to repeat the godown experiments using wild Bombay rats instead of guinea-pigs.

SERIES I.

The godowns used in this series were Nos. 1, 2, 5 and 6. It will be remembered that godowns Nos. 1 and 2 had roofs of country tiles which afforded protection to wild rats. Hence in these godowns there was a more or less abundant supply of rat fleas. The roofs of godowns Nos. 5 and 6 were of reinforced concrete which of course offered no shelter to wild rats.

Early in February 1908 it was ascertained by guinea-pig tests that godowns Nos. 1 and 2 contained a large number of fleas, while Nos. 5 and 6 were free from fleas. 25 healthy rats were then introduced into each of the godowns. These rats were wild Bombay *Mus rattus* which had been kept in captivity for several weeks before being put into the godowns. It was necessary to remove the fleas from the rats intended for godowns Nos. 5 and 6. This was done by immersing the rats momentarily in an emulsion of "Hydrocarbon" (a by-product in the manufacture of Pirsch's oil gas containing a considerable percentage of benzene). After immersion the rats were kept for some days in cages suspended above the ground until they had recovered from the effects of their immersion. Before being put in godowns Nos. 5 and 6 a certain number of them were examined for fleas. This method of removing fleas from the rats appeared to be effectual as we were never able to discover any fleas on the rats that had been dipped and subsequently kept in suspended cages. The mortality among the rats as a result of the immersion was however very high. A considerable number of rats died in the godowns, especially Nos. 5 and 6, during the week following their introduction. These were replaced by fresh rats.

The following table shows the number of fleas caught on rats in each godown on several occasions before and during the progress of the experiments:

Flea counts in godowns.

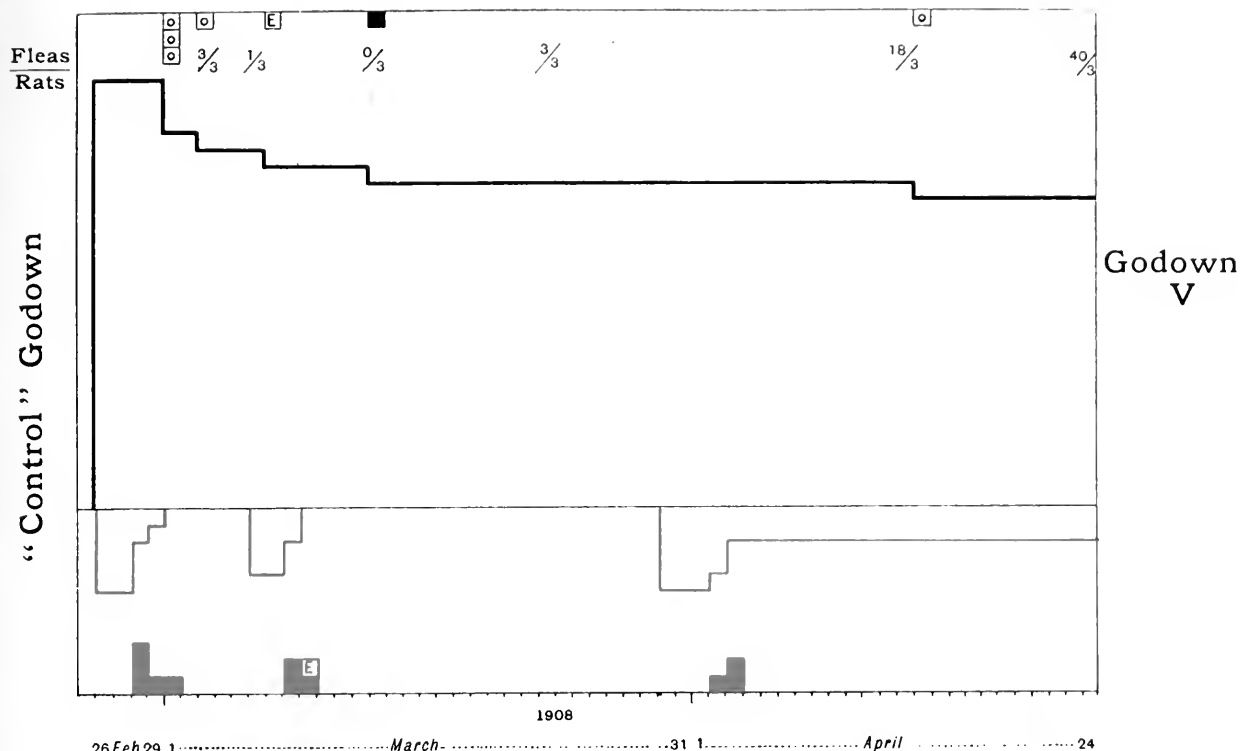
Date	Godown No. 1		Godown No. 2		Godown No. 5		Godown No. 6	
	No. of rats examined	No. of fleas	No. of rats examined	No. of fleas	No. of rats examined	No. of fleas	No. of rats examined	No. of fleas
23. II. 08	6	120	6	76	8	0	8	0
3. III. 08	3	49	2	13	3	3	2	1
6. III. 08	—	—	—	—	3	1	3	0
13. III. 08	2	22	2	31	3	0	3	2
23. III. 08	3	56	3	49	3	3	3	5
13. IV. 08	3	30	3	31	3	18	3	9
24. IV. 08	—	—	—	—	3	49	3	48

Note:—Fleas caught on rats in godowns Nos. 1 and 2 were subsequently returned to the godowns.

Experiment I. Diagram I.

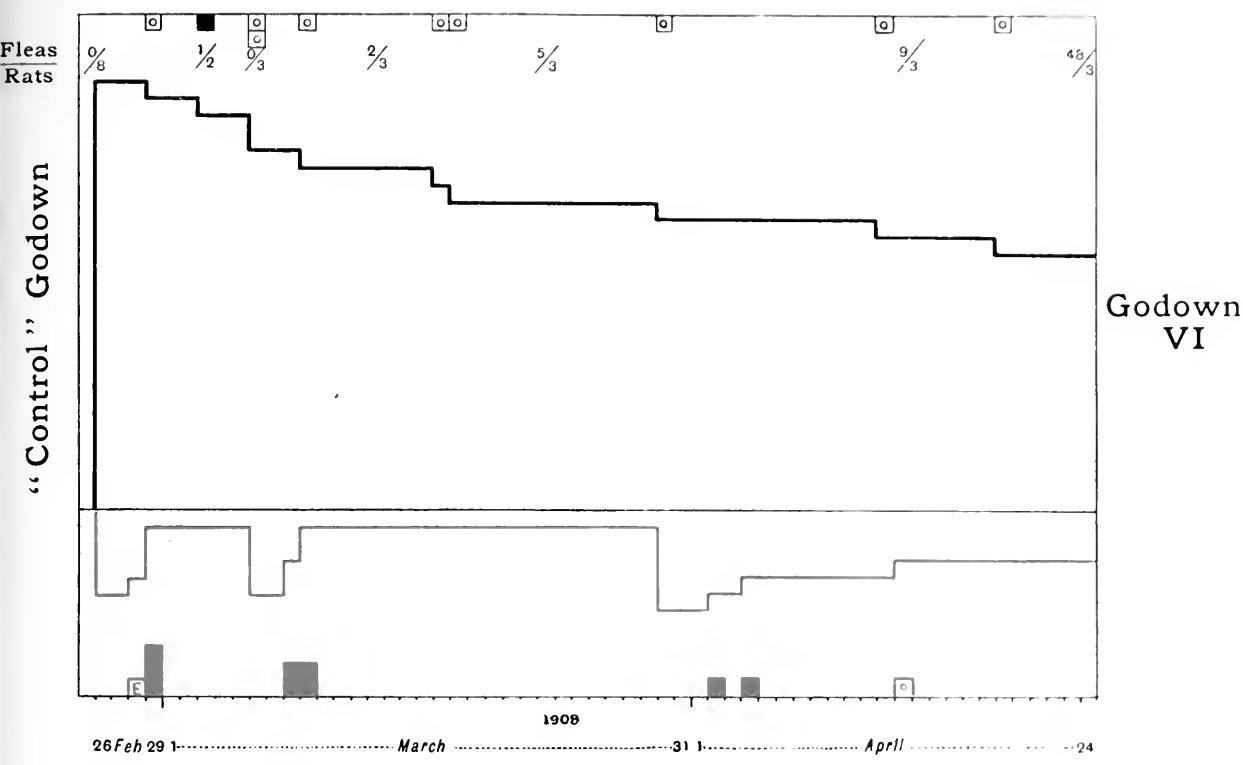
It will be seen that quite early in the experiment fleas were present in small numbers in the control godown No. 5. The number of fleas increased considerably in April. Of 12 rats proved to have died of

DIAGRAM I



- number of un inoculated rats alive.
- number of inoculated rats alive.
- un inoculated
 - inoculated
 } rat dead of plague.
- un inoculated
 - inoculated
 } rat dead, not plague.
- ⓔ un inoculated
 - ⓔ inoculated
 } rat dead, but eaten too much to ascertain cause of death.

DIAGRAM II



- number of uninoculated rats alive.
- number of inoculated rats alive.
- uninoculated
 - inoculated } rat dead of plague.
- uninoculated
 - inoculated } rat dead, not plague.
- E uninoculated
 - E inoculated } rat dead, but eaten too much to ascertain cause of death.

plague, the carcasses of nine were more or less eaten. Only one uninoculated rat died of plague and it showed no signs of an intestinal infection.

In the flea-infested godown No. 1 uninoculated rats died of plague on the same day as the first inoculated rats died. This suggests that the godown was previously infected from wild rats living in the tiles of the roof (cf. godown 2, Experiment II). On April 9th five more uninoculated rats were put in the godown. Of these one died of plague on April 18th, showing that the godown was infective, though none of the survivors of the original epizootic became infected. This suggests that the survivors were immune.

The chief value of the experiment is that it shows that feeding may take place to a considerable extent without giving rise to an epizootic.

Experiment I. Feb. 26th to April 24th.

	Inoculated rats		Uninoculated rats		Plague rats	Flea counts,
	Put in	Died of plague	Put in	Died of plague	eaten	average per rat
Godown 5	14	11	25	1	9	3·6
Godown 1	10	8	30	13	1	17

Experiment II. Diagram II.

In the control godown No. 6 fleas were present in small numbers and the number increased in April. In this godown only one out of 11 rats proved to have died of plague was eaten.

With reference to the heavy mortality from causes other than plague among the uninoculated rats in this godown, it may be remarked that two of them died from the effects of chloroform when being searched for fleas and several were suffering from ulcerations due to having been dipped in "Hydrocarbon" emulsion.

In godown 2 infection was started from wild rats living in the roof. As in the previous experiment we failed to infect any of the survivors of the epizootic by putting in a fresh lot of inoculated rats. None of the ten rats which died of plague in this godown were eaten.

Experiment II. Feb. 26th to April 24th.

	Inoculated rats		Uninoculated rats		Plague rats	Average
	Put in	Died of plague	Put in	Died of plague	eaten	fleas per rat
Godown 6	14	9	25	1	1	2·5
Godown 2	[5]	[2]	23	8	0	12·5

SERIES II.

In these experiments a series of new godowns were used, numbered 7 to 12. They were built throughout of reinforced concrete; there was therefore no natural supply of fleas from rats living in the roofs. The flea-infected godowns were deliberately stocked with fleas, about 400 fleas being put each week into each of the godowns 10, 11 and 12. For about six weeks the numbers found remained small, but later on they became abundant, presumably because breeding had started. In other respects the experiments of this series were done in the same way as those in Series I.

Experiment III. Diagram III.

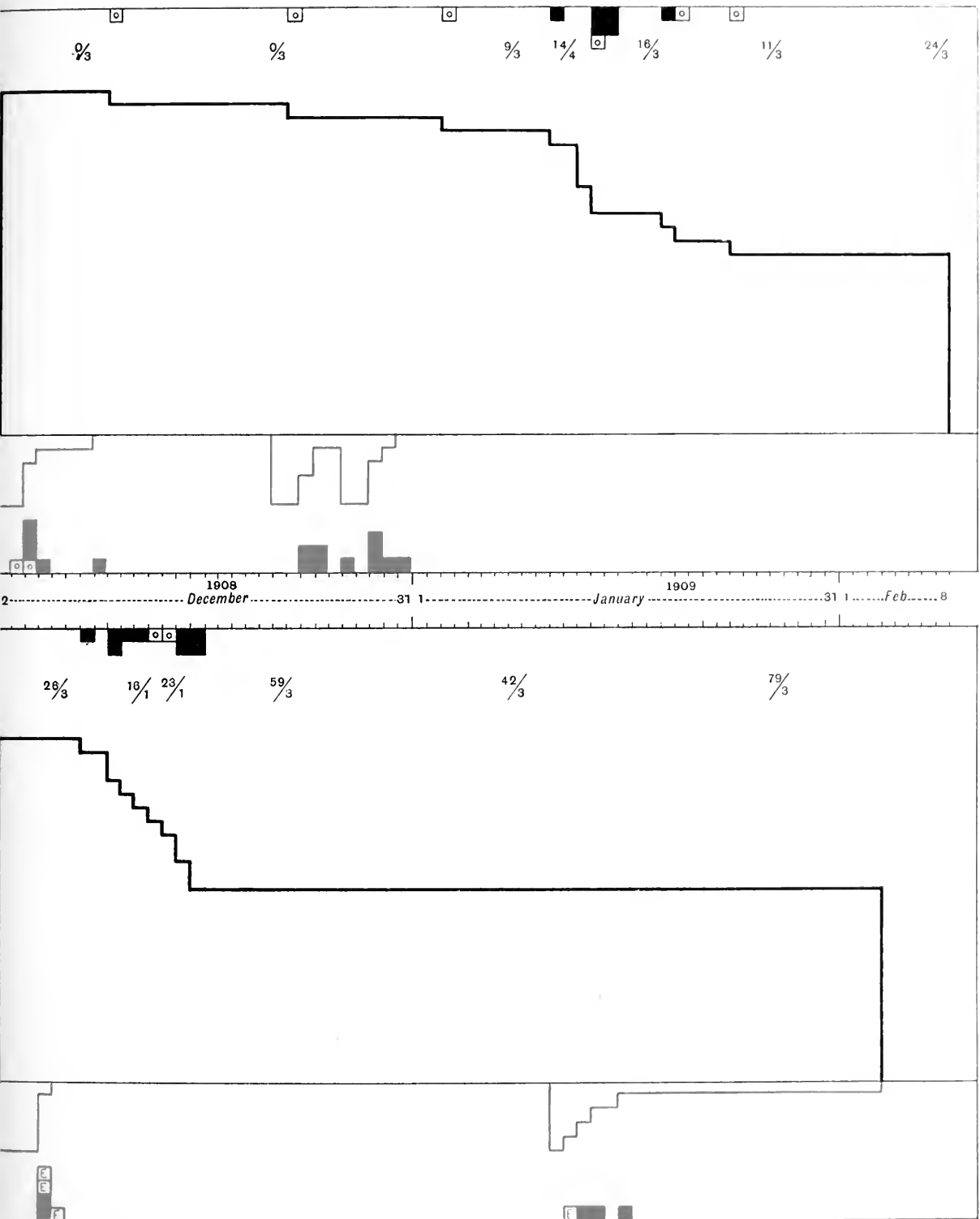
No deaths from plague occurred among the uninoculated rats in godown No. 7 as long as it remained free from fleas, although 15 of the inoculated rats died from plague. Five weeks after the commencement of the experiment a flea count showed that fleas were now present in the godown in small numbers and a few days later the uninoculated rats began to die. Of the 21 rats that died of plague in this godown none were eaten. On Feb. 8 the surviving rats were inoculated with a dose of an emulsion of a plague rat's spleen (= 1/1000 grain of spleen): 4 of the 12 (33 %) died of plague, while of 20 control rats inoculated at the same time 95 % died of plague.

In the flea-infested godown No. 10, nine of the uninoculated rats died of plague. The carcasses of four inoculated rats and seven uninoculated were found to have been eaten, several almost completely. Of the nine uninoculated rats which were proved to have died of plague, two had definite mesenteric buboes, three had no buboes and the remaining four had been eaten to such an extent that the presence or absence of a bubo could not be determined. It is evident then that at least two of these rats were infected by feeding.

An attempt to re-start the epizootic by putting in infected rats and six infected guinea-pigs failed.

On Feb. 3rd the surviving rats were removed to godown No. 12 which was at the time highly infective for guinea-pigs and were kept there for two weeks. None of them contracted plague.

DIAGRAM III



- number of uninoculated rats alive.
- number of inoculated rats alive.
- uninoculated
 - inoculated
 } rat dead of plague.
- uninoculated
 - inoculated
 } rat dead, not plague.
- ⌈ uninoculated
 - ⌈ inoculated
 } rat died, but eaten too much to ascertain cause of death.

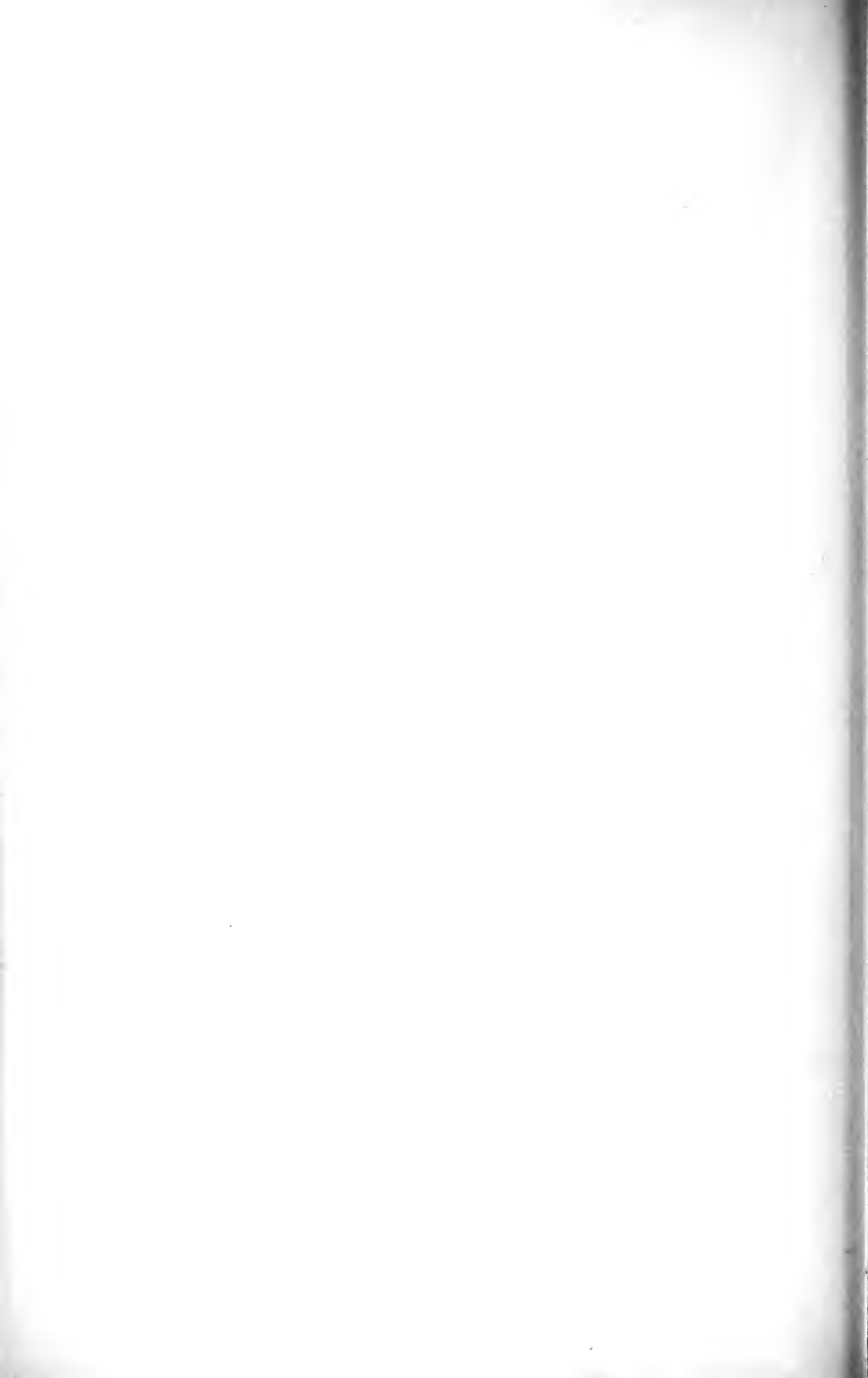
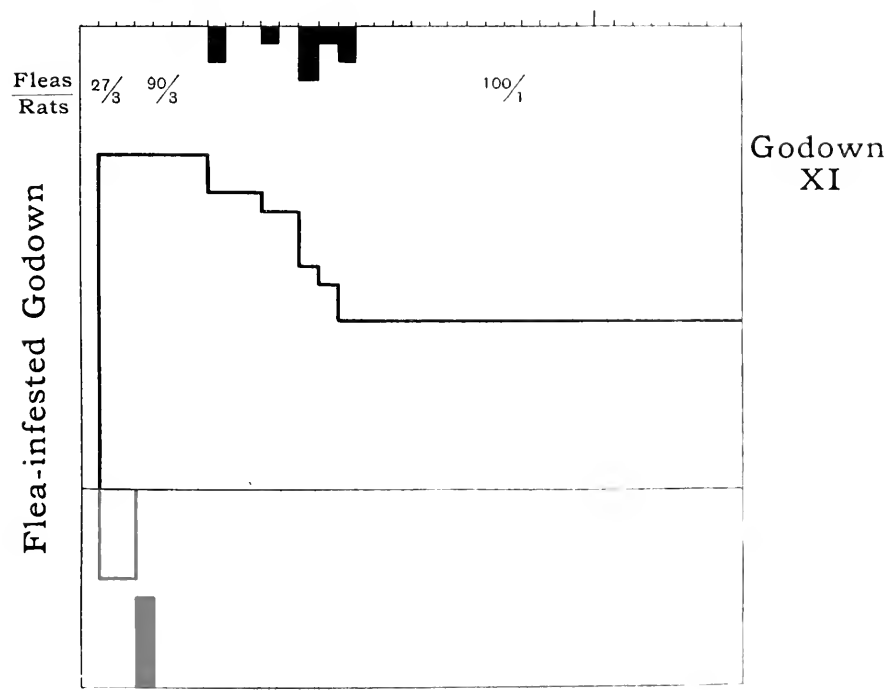
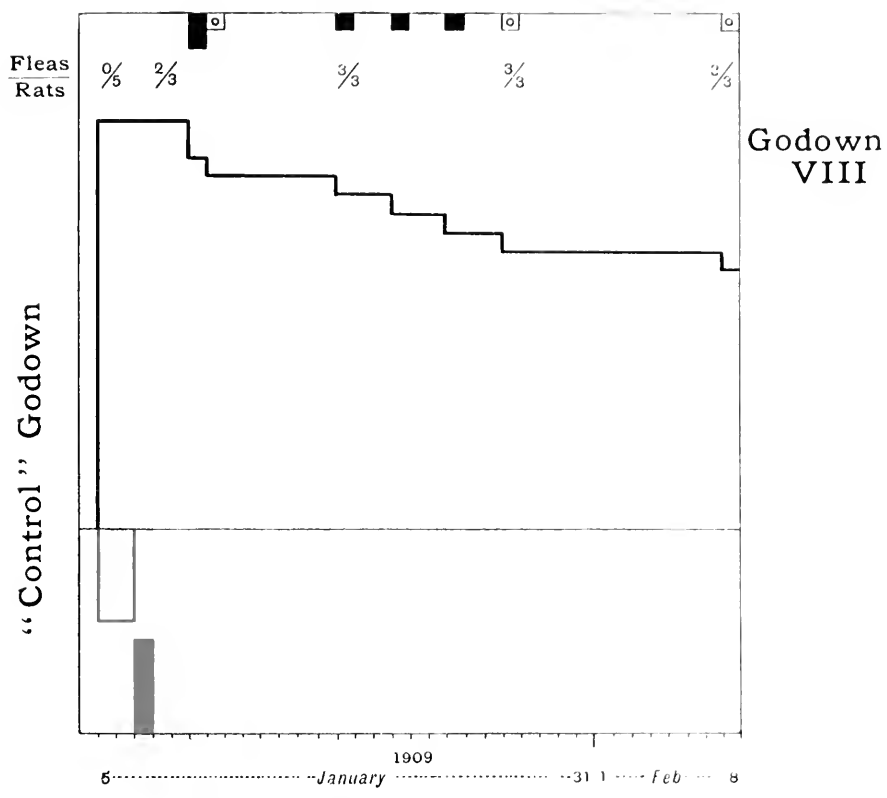


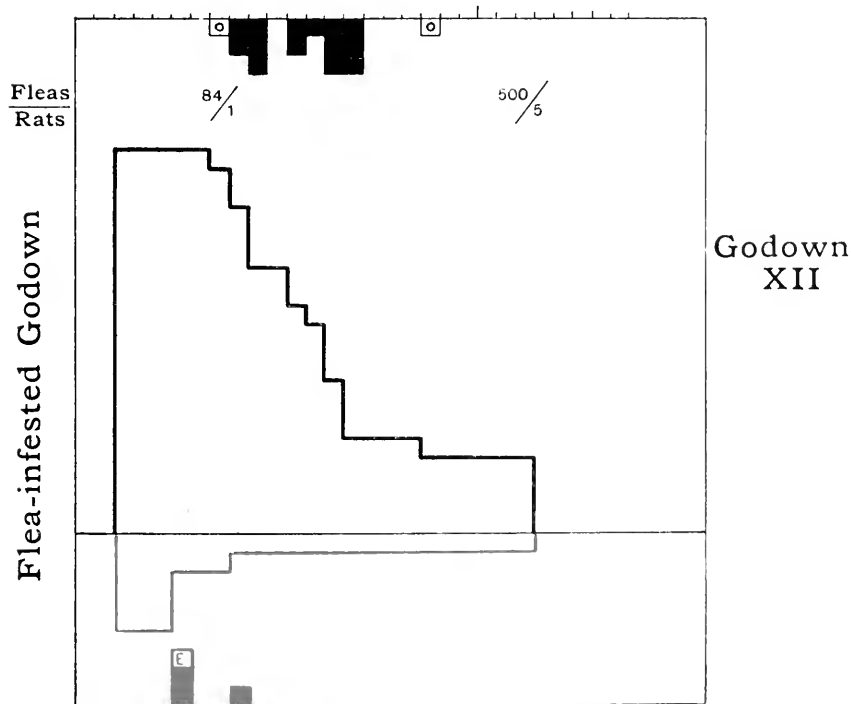
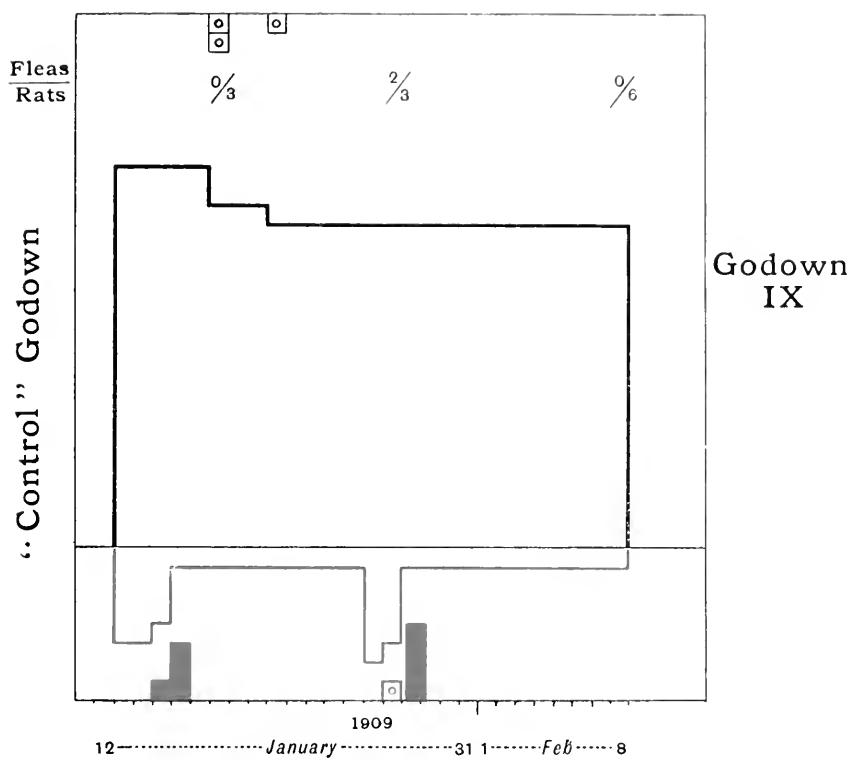
DIAGRAM IV



- number of uninoculated rats alive.
- number of inoculated rats alive.
- uninoculated } rat dead of plague.
- inoculated }
- uninoculated } rat dead, not plague.
- inoculated }
- ⊞ uninoculated } rat dead, but eaten too much
- ⊞ inoculated } to ascertain cause of death.



DIAGRAM V



- number of uninoculated rats alive.
- number of inoculated rats alive.
- uninoculated
 - inoculated } rat dead of plague.
- uninoculated
 - inoculated } rat dead, not plague.
- ⊞ uninoculated
 - ⊞ inoculated } rat dead, but eaten too much to ascertain cause of death.

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On Feb. 18th the 14 surviving uninoculated rats were inoculated subcutaneously with a dose of an emulsion of the spleen of a plague rat (=1/5000 grain of spleen). None of these rats died of plague, whereas of 30 control rats which were inoculated at the same time, 60% died of plague.

Experiment III. Dec. 2nd to Feb. 8th.

	Inoculated rats		Uninoculated rats		Plague rats	Average
	Put in	Died of plague	Put in	Died of plague	eaten	fleas per rat
Godown 7	17	15	25	6	0	3.5
Godown 10	10	5	25	9	4	17.5

Experiment IV. Diagram IV.

Fleas were present in the control godown, No. 8, in small numbers throughout the experiment. The epizootic consisted of five cases spread over a fortnight. None of the rats that died in this godown were eaten. On Feb. 8, ten of the surviving rats were inoculated with the equivalent of 1/1000 grain of the spleen of a plague-infected rat. Of these eight (80%) died of plague, while of 20 control rats 95% died of plague.

In the flea-infested godown No. 11, two of the 14 rats that died of plague were partially eaten. The immunity of the surviving rats was tested by subcutaneous inoculation on Feb. 18th. Of nine rats which received the equivalent of 1/5000 grain of the spleen of a plague rat, none died, whereas of 30 control rats 60% died.

This experiment illustrates the more rapid course of the epizootic in the godown with a higher flea infestation.

Experiment IV. Jan. 5th to Feb. 8th.

	Inoculated rats		Uninoculated rats		Plague rats	Average
	Put in	Died of plague	Put in	Died of plague	eaten	fleas per rat
Godown 8	5	5	22	5	0	0.7
Godown 11	5	5	18	9	2	31

Experiment V. Diagram V.

In this experiment special precautions were taken to render the control godown, No. 9, as far as possible flea-free. Immediately before commencing the experiment the rats already in the godown were removed and again freed from fleas under chloroform before being put

back. While the rats were out the godown was cleaned and washed out with pure kerosine oil. The control godown remained practically free from fleas throughout the experiment. None of the rats which died were eaten. On Feb. 8th the surviving rats were inoculated subcutaneously with the equivalent of 1/1000 grain of spleen of a plague rat. Of 16 thus inoculated 15 (= 94 %) died of plague, while of 20 controls 95 % died of plague.

In the flea-infested godown, No. 12, the number of fleas was far in excess of that observed in other experiments. Of 17 rats dead of plague three were eaten,—one almost completely. None of the 14 uninoculated rats that died of plague showed signs of an intestinal infection. On Feb. 3rd four uninoculated rats which remained alive were inoculated with the equivalent of 1/1000 grain of the spleen of a plague rat. None of these died of plague, while of two control rats one died of plague.

This experiment illustrates well the rapid and extensive spread of plague among the rats in the presence of very large numbers of fleas.

Experiment V. Jan. 12th to Feb. 8th.

	Inoculated rats		Uninoculated rats		Plague rats	Average
	Put in	Died of plague	Put in	Died of plague	eaten	fleas per rat
Godown 9	10	8	20	0	0	0·2
Godown 12	5	3	20	14	3	97

Experiment VI. Diagram VI.

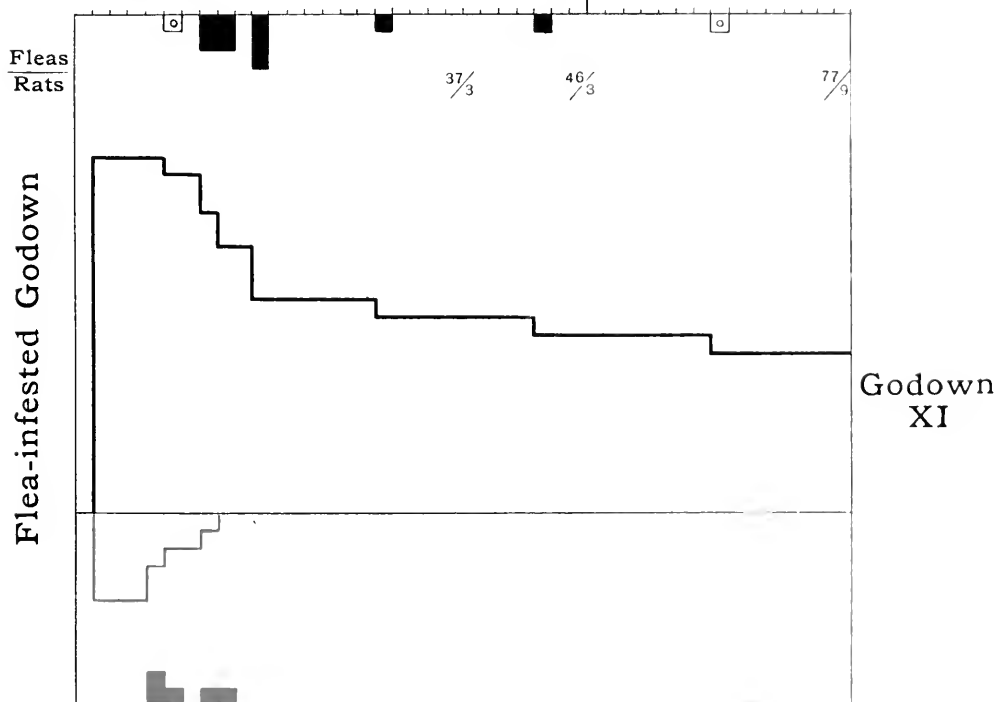
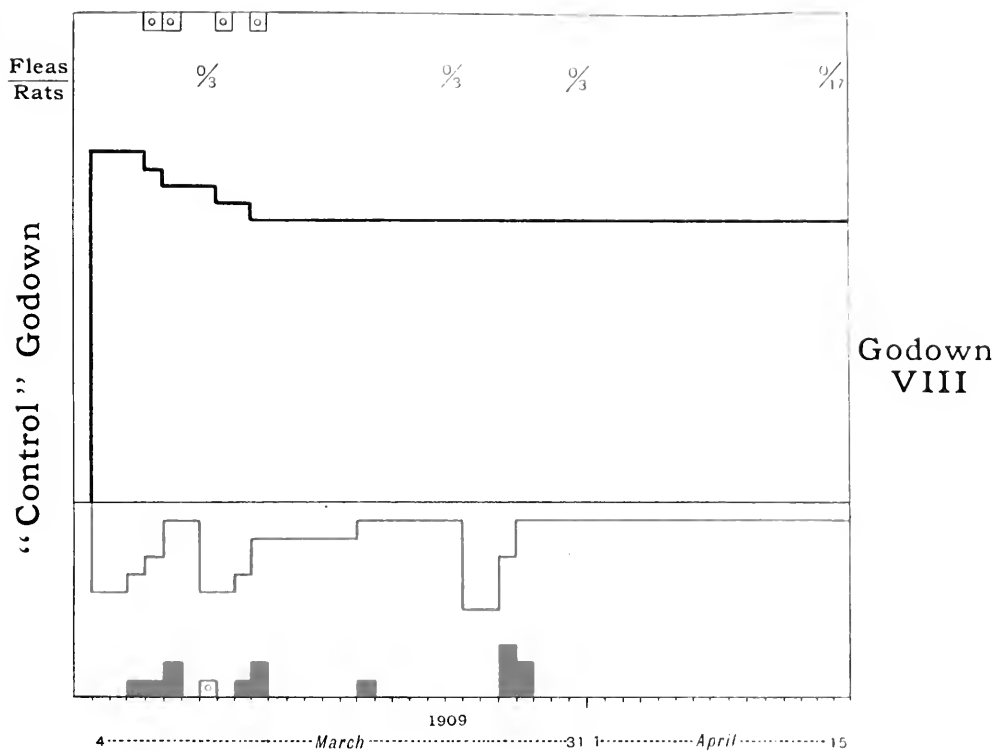
The control godown, No. 8, remained free from fleas throughout. None of the rats that died in this godown were eaten. On April 15th sixteen rats remaining in the godown were inoculated subcutaneously with the equivalent of 1/2000 grain of a spleen of a plague-infected rat. Of this number seven (47 %) died of plague, as compared with 40 % of 23 controls.

None of the rats that died in the flea-infested godown No. 11 were eaten. Of nine survivors inoculated with the same dose of spleen as the rats in the control godown none died of plague.

Experiment VI. March 4th to April 15th.

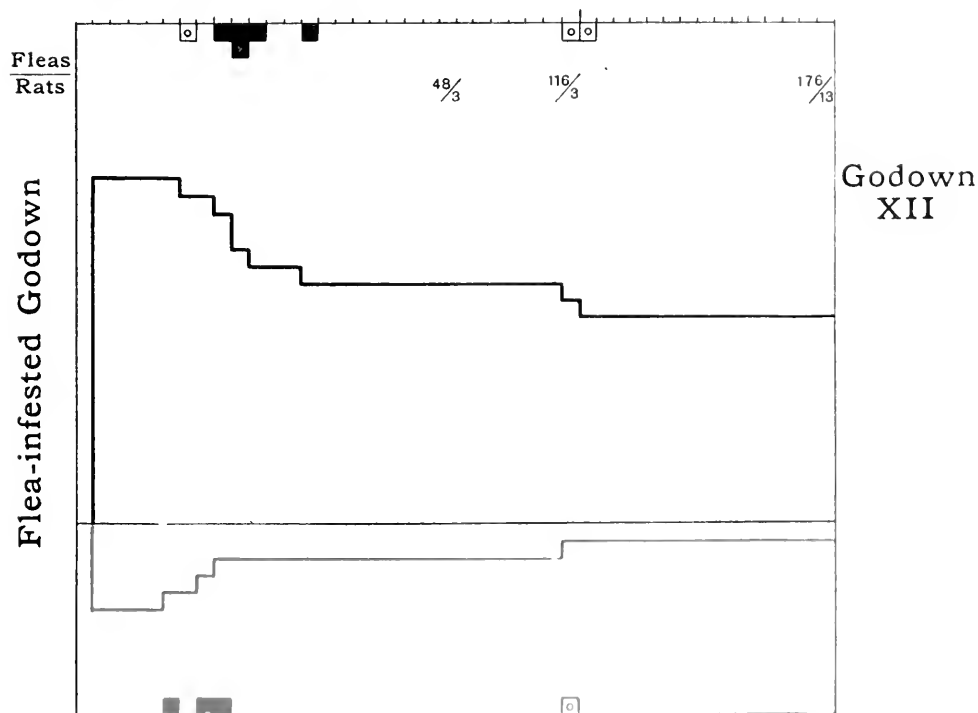
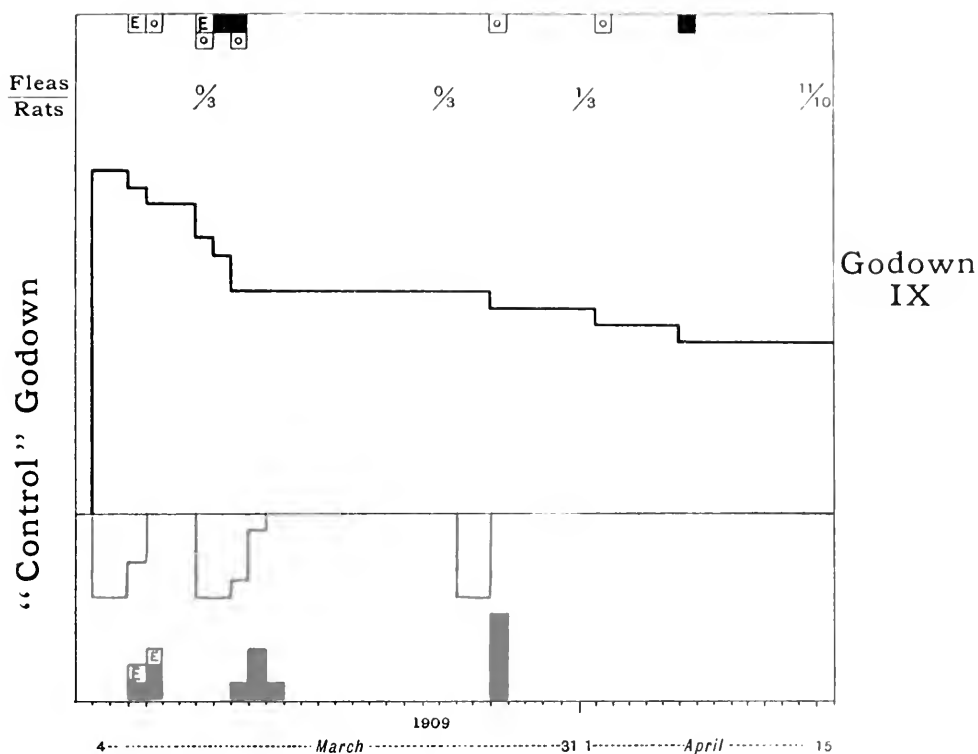
	Inoculated rats		Uninoculated rats		Plague rats	Average
	Put in	Died of plague	Put in	Died of plague	eaten	fleas per rat
Godown 8	15	13	20	0	0	nil
Godown 11	5	5	20	9	0	10·5

DIAGRAM VI



- number of uninoculated rats alive.
- number of inoculated rats alive.
- uninoculated
 - inoculated
 } rat dead of plague.
- uninoculated
 - inoculated
 } rat dead, not plague.
- ⓔ uninoculated
 - ⓔ inoculated
 } rat dead, but eaten too much to ascertain cause of death.

DIAGRAM VII



- number of uninoculated rats alive.
- - - number of inoculated rats alive.
- uninoculated
 - inoculated } rat dead of plague.
- uninoculated
 - inoculated } rat dead, not plague.
- ⊞ uninoculated
 - ⊞ inoculated } rat dead, but eaten too much to ascertain cause of death.

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Experiment VII. Diagram VII.

In control godown, No. 9, many of the carcasses were eaten. Of the first five inoculated rats put in and that died, two were almost completely and one partially eaten, while two of the second five were partially eaten. Of the uninoculated rats that died, two were eaten to such an extent that the cause of death could not be determined, and two of the three rats that were proved to be plague-infected were partially eaten.

On April 15th nine rats from this godown were inoculated with 1/2000 of a grain each of the spleen of a plague-infected rat: three of them died of plague, as compared with 40 % of the controls.

Experiment VII. March 4th to April 15th.

	Inoculated rats		Uninoculated rats		Plague rats	Average
	Put in	Died of plague	Put in	Died of plague	eaten	fleas per rat
Godown 9	15	13	20	3	9	0·6
Godown 12	5	3	20	5	0	18

SERIES III.

In this series the new godowns, 7 to 12, were used. The technique differed from that previously used in three particulars. In the first place the godowns were further protected from fleas by building a gutter filled with water about 6 inches wide in front of the doorways. In the second place the godowns were more effectively cleared of fleas before the experiments commenced by fumigating them with hydrocyanic acid vapour after the washing with kerosine emulsion which had alone been previously employed; eggs and larvae are killed by this method as well as adult fleas. The third point of difference to be noted is that the inoculated rats which were introduced were confined in cages, instead of running loose, on the floor of the godowns: this did not altogether prevent their carcasses being partially eaten. The absence of fleas in the control godowns was controlled by guinea-pig flea traps. The rats were cleared of fleas by dipping in "Hydrocarbon" as before; they were then hung up for a week or two where they were inaccessible to fleas and finally examined under chloroform to make sure that they were free from fleas before being put into the flea-free godowns. In this way we succeeded in keeping a number of godowns almost if not quite free from fleas for considerable periods.

*Experiment VIII.**A. Control Godowns.*

Godown No. 7. This godown failed as control as it became flea infested almost from the start. A death from plague among the uninoculated rats occurred in the first week and a flea found on the rat which died of plague showed abundant *B. pestis* in the stomach.

Godown No. 8. Fifteen uninoculated rats and five rats inoculated with a virulent emulsion of a plague guinea-pig spleen were put in on Sept. 24. These rats were all flea-free. Four of the inoculated rats died of plague by Sept. 29. One died from some other cause. A second lot of five inoculated rats were put in on Oct. 13. All died of plague on Oct. 15. The experiment was continued until Nov. 2, and during the whole period no death from plague occurred among the fifteen uninoculated rats. A weekly flea-count showed the godown to be flea-free throughout. None of the rats which died in this godown were eaten.

Godown No. 9. Fifteen uninoculated rats and five inoculated rats were put into this godown on Sept. 24. Three of the inoculated rats died of plague by Sept. 30: one died of some other cause and one survived. A second lot of five inoculated rats was introduced on Oct. 13. All of these died of plague by Oct. 16. The godown remained flea-free throughout until Nov. 2 when one flea was found and the experiment terminated. No death from plague among the uninoculated rats occurred in this godown. No rats which had died of plague in this godown were eaten.

B. Flea Godowns.

Godown No. 10. Fifteen uninoculated rats and five rats inoculated with the same emulsion as was used to infect the rats in the control godowns were introduced on Sept. 24. All five inoculated rats were dead from plague by Sept. 29. Five more inoculated rats were put in on Oct. 13: these were all dead of plague by Oct. 22. One uninoculated rat died of plague on Oct. 6 and another on Oct. 14. The average of the weekly number of fleas present during this experiment was 2.5 per rat. None of the rats dead of plague in this godown were eaten.

Godown No. 11. Five inoculated and fifteen uninoculated rats put in on Sept. 24. Four inoculated rats were dead of plague by Sept. 28:

one survived. Five more inoculated rats were put in on Oct. 13. Four of these were dead of plague by Oct. 17, one survived.

One uninoculated rat died of plague on Sept. 27, a second on Oct. 26, and a third on Nov. 2. The average number of fleas for the period was 1.4 per rat. No plague rats eaten.

Godown No. 12. Five inoculated and fifteen uninoculated rats were introduced on Sept. 24. Three inoculated rats died of plague by Sept. 30 : one died less acutely of plague on Oct. 9 : one survived.

Five more inoculated rats were put in on Oct. 13 : four of these died of plague by Oct. 17, and one from some other cause. No death from plague occurred among the uninoculated rats. Average of fleas per rat for the period was seven per rat. One plague rat eaten.

Experiment VIII. Sept. 24th to Nov. 2nd.

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	10	9	15	1	0	1 found on Sept. 27 contained <i>B. pestis</i> in its stomach.
No. 8	10	9	15	0	0	Nil.
No. 9	10	8	15	0	0	Nil.
No. 10	10	10	15	2	0	Average 2.5 per rat.
No. 11	10	8	15	3	0	Average 1.4 per rat.
No. 12	10	8	15	0	1	Average 7 per rat.

Experiment IX.

A. *Control Godowns.*

Of these godowns only No. 9 remained perfectly flea-free, one flea being found in No. 7 and one in No. 8 during the course of the experiment.

Godown No. 7. Five inoculated and fifteen uninoculated rats were put in on Nov. 5. Four inoculated rats were dead of plague by Nov. 10 : one survived. Four more inoculated rats were put in on Nov. 17, three of which died of plague by Nov. 19 and the fourth died of some other cause. A third lot of five inoculated rats was introduced on Nov. 26, three of which died of plague by Nov. 29 : two survived. No deaths from plague occurred among the uninoculated rats. One flea was found in the godown on Nov. 15 and removed. No more fleas were found until Dec. 13, when one was found and the experiment stopped. No plague rats were eaten.

Godown No. 8. Five inoculated and fifteen uninoculated rats were put in on Nov. 5. Three of the inoculated rats were dead of plague by Nov. 10: two survived. Five more inoculated rats were put in on Nov. 17, all of which were dead of plague by Nov. 20. A third lot of five was introduced on Nov. 26, all of which died of plague by Dec. 3. The total number of inoculated rats dead of plague in the godown is thus thirteen. Of the fifteen uninoculated rats exposed to infection none died of plague. One flea was found in the godown on Nov. 29 and removed. When the experiment was terminated on Dec. 13 the godown was flea-free. One plague rat was partly eaten during this experiment.

Godown No. 9. Five inoculated and fifteen uninoculated rats were put in on Nov. 5. Three of the inoculated rats were dead of plague by Nov. 9 and a fourth died of plague on Nov. 16: one survived. Four more inoculated rats were introduced on Nov. 17, all of these died of plague by Nov. 21. A third lot of five inoculated rats was put in on Nov. 26; four of these died of plague by Dec. 1, one survived. The total number of inoculated rats which died of plague in this godown was 12. None of the fifteen uninoculated rats developed plague. This godown remained flea-free until the termination of the experiment. No plague rats eaten.

B. *Flea Godowns.*

Godown No. 10. Five inoculated and fifteen uninoculated rats were put in on Nov. 5. All the inoculated rats died of plague by Nov. 9. A second lot of five inoculated rats was put in on Nov. 25: all of these died of plague by Nov. 30. One uninoculated rat died of plague on Nov. 14, a second one on Nov. 18, a third on Nov. 20, a fourth on Nov. 22, and a fifth on Nov. 29. The average number of fleas present for the period was 13 per rat. No plague rats eaten.

Godown No. 11. Five inoculated and 15 uninoculated rats were put in on Nov. 5. Four of the inoculated rats died of plague by Nov. 8: one survived. Five more inoculated rats were put in on Nov. 26: all of these died of plague by Dec. 1. Deaths from plague among the uninoculated rats occurred on the 13th, 14th, 18th, 20th, and 22nd November—five in all. The average flea count during the epizootic was 18 per rat. Four rats which died from plague in this godown were partially eaten.

Godown No. 12. Five inoculated and fifteen uninoculated rats were introduced on Nov. 5. All five inoculated rats were dead of

plague by Nov. 8. Five more inoculated rats were put in on Nov. 26: all died of plague by Nov. 30. Three uninoculated rats died of plague on Nov. 14, two on Nov. 15, one on Nov. 18 and one on Nov. 20. Average number of fleas present during the period was 39 per rat. No plague rats were eaten in this godown.

Experiment IX. Nov. 5th to Dec. 13th.

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	14	10	15	0	0	One flea found on Nov. 15.
No. 8	15	13	15	0	1	One flea found on Nov. 29.
No. 9	14	12	15	0	0	Nil.
No. 10	10	10	15	5	0	Average 13 per rat.
No. 11	10	9	15	5	4	Average 18 per rat.
No. 12	10	10	15	7	0	Average 39 per rat.

Experiment X.

A. Control Godowns.

Godown No. 7. Five inoculated and fifteen uninoculated flea-free rats were put in on Dec. 21. All the inoculated rats were dead of plague by Dec. 24. The godown remained flea-free until Jan. 17 when it was found flea infested. No deaths from plague occurred among the uninoculated rats, the last plague death in the godown having occurred three weeks before it became flea infested. No plague rats were eaten in this godown.

Godown No. 8. Five inoculated and fifteen uninoculated rats were put in on Dec. 21. All the inoculated rats were dead of plague by Dec. 24. None of the uninoculated rats died of plague. The godown remained flea-free until the termination of the experiment on Jan. 24. One plague rat in this godown was partially eaten.

Godown No. 9. Five inoculated and fifteen uninoculated flea-free rats were introduced on Dec. 24. Four inoculated rats were dead of plague and one from some other cause by Dec. 26. The godown remained flea-free throughout and there were no deaths from plague among the uninoculated rats. Two plague rats in the godown were partially eaten.

B. *Flea Godowns.*

Godown No. 16. Five inoculated and fifteen uninoculated rats were put in on Dec. 21. All the inoculated rats died of plague by Dec. 23. The first plague death among the uninoculated rats occurred on Dec. 28. Between that date and Jan. 3, seven more died of plague. Another death occurred on Jan. 10 and the last one on Jan. 17. Ten out of the fifteen uninoculated rats exposed to infection in this godown thus died of plague. The flea count during the progress of this epizootic was 34 per rat. One rat which died of plague in this godown was partially eaten.

Godown No. 11. Five inoculated and fifteen uninoculated rats were put in on Dec. 21. All five inoculated rats were dead of plague by Dec. 24. The first plague death among the uninoculated rats occurred on Dec. 30. Four more deaths occurred on the four succeeding days and a last one on Jan. 6; the total number of uninoculated rats dying of plague was six. The flea count for the period was 29 per rat.

Godown No. 12. Five inoculated and fifteen uninoculated rats were introduced on Dec. 21. All five inoculated rats were dead of plague by Dec. 24. Three uninoculated rats died of plague on Dec. 25, four more on Dec. 26, three on Dec. 27 and one on Jan. 1st, the total number of rats succumbing to the epizootic being 11 out of the 15. The flea count during the experiment averaged 48 per rat. No plague rats were eaten in this godown.

Experiment X. Dec. 21st to Jan. 24th.

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	5	5	15	0	0	Godown flea infested on Jan. 17.
No. 8	5	5	15	0	1	Nil.
No. 9	5	4	15	0	2	Nil.
No. 10	5	5	15	10	1	Average 34 per rat.
No. 11	5	5	15	6	0	Average 29 per rat.
No. 12	5	5	15	11	0	Average 48 per rat.

*Experiment XI.*A. *Control Godowns.*

Godown No. 7. Fifteen uninoculated rats and five rats inoculated with a virulent emulsion of plague rat's spleen were put into this godown on Feb. 3. Four of the inoculated rats died of plague by

Feb. 7. Five more inoculated rats were introduced on Feb. 25, four of which died of plague by Feb. 28. The experiment was continued till March 3, and the godown remained flea-free throughout. No deaths from plague occurred among the uninoculated rats. None of the rats dead of plague were eaten.

Godown No. 8. Fifteen uninoculated and five inoculated rats were put in on Feb. 3. All the inoculated rats died of plague by Feb. 8. Five more inoculated rats were put in on Feb. 25, four of which died of plague by Feb. 28. The godown remained flea-free until March 7, when one flea was found. The experiment was terminated three days later. No deaths from plague occurred among the uninoculated rats. No plague rats were eaten.

Godown No. 9. Fifteen uninoculated and five inoculated rats were put in on Feb. 3. Three of the inoculated rats died of plague by Feb. 6; two survived. A second lot of five inoculated rats was put in on Feb. 25, all of which died on Feb. 27. The godown remained flea-free until March 7 when two fleas were found. The experiment was terminated three days later. No deaths from plague occurred among the uninoculated rats in this godown. No plague rats were eaten.

B. *Flea Godowns.*

Godown No. 10. Fifteen uninoculated rats and five rats inoculated with the same emulsion as was used in the flea-free godowns were introduced on Feb. 3. Only two of the inoculated rats died of plague (on Feb. 5 and 6), the other three surviving. The first uninoculated rat which developed plague died on Feb. 9. Another died on Feb. 10, and seven more between Feb. 12 and 16: nine deaths in all. Five more inoculated rats were introduced on Feb. 25, all of which died of plague by Feb. 28. No more uninoculated rats developed plague after the introduction of this second lot of inoculated rats. No plague rats were eaten. The average flea count for the period was 20 per rat. After the experiment ended on March 10, three rats were put in to provide food for the fleas; none died of plague.

Godown No. 11. Fifteen uninoculated and five inoculated rats were put in on Feb. 3. Four of the inoculated rats died of plague by Feb. 12: the fifth rat lived until Feb. 23 and died of subacute plague. One uninoculated rat died of plague on Feb. 6, the day after the first two inoculated rats died. Other deaths of uninoculated rats occurred on Feb. 9, 13, 14 and 20. Five more inoculated rats were introduced on

Feb. 25: two of these died of plague on Feb. 27, and one on Feb. 28. One more uninoculated rat died on Feb. 27. The total number of uninoculated rats dying of plague is thus six, five of which deaths occurred before the introduction of the second lot of inoculated rats. The flea count for the period averaged 14 per rat. One plague rat was partly eaten. After the experiment ended on March 10, three fresh rats were put in; one died of plague during the following week.

Godown No. 12. Fifteen uninoculated and five inoculated rats were put into this godown on Feb. 3. Four inoculated rats died of plague by Feb. 7. One uninoculated rat died of plague on Feb. 8, two on Feb. 11, and a fourth on Feb. 12. A second lot of five inoculated rats was put in on Feb. 25, all of which died of plague by March 1. No more uninoculated rats died of plague. The flea count during the progress of the experiment averaged 15 fleas per rat. No plague rats were eaten. After the experiment ended on March 10, three fresh rats were put in: two died of plague during the following week.

Experiment XI. Feb. 3rd to March 10th.

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	10	8	15	0	0	Nil.
No. 8	10	9	15	0	0	One flea found on March 7.
No. 9	10	8	15	0	0	Two fleas found on March 7.
No. 10	10	7	15	9	0	Average 20 fleas per rat.
No. 11	10	8	15	6	1	Average 14 fleas per rat.
No. 12	10	9	15	4	0	Average 15 fleas per rat.

Experiment XII.

A. Control Godowns.

Godown No. 7. Fifteen uninoculated rats and five rats inoculated with an emulsion of a plague rat spleen were put in on March 22. The five inoculated rats died of plague on March 24. Five more were introduced on April 7, all of which died of plague by April 16, and a third lot of five put in on April 19 all died of plague on April 21. The godown remained flea-free until April 25 when one flea was found. The

experiment was terminated a week later, no deaths from plague having occurred among the uninoculated rats. No plague rats were eaten.

Godown No. 8. Fifteen uninoculated flea-free rats and five inoculated rats were put in on March 22. All five inoculated rats were dead of plague by March 25. Five more inoculated rats were put in on April 7, four of which died of plague by April 11. A third lot of five was introduced on April 19 all of which died of plague on April 21. The godown remained flea-free throughout and none of the uninoculated rats died of plague. The experiment was terminated on May 2nd. No plague rats were eaten.

Godown No. 9. Fifteen uninoculated and five inoculated rats were put in on March 22. The five inoculated rats died of plague by March 29. Five more inoculated rats were put in on April 7, four of which died by April 19. A third lot of five was introduced on April 19, all of which died of plague on April 21. The godown remained flea-free until April 25, when four fleas were found. No plague rats were eaten.

B. *Flea Godowns.*

Godown No. 10. Fifteen uninoculated rats and five rats inoculated with the same emulsion as those in the control godowns were put in on March 22. Three of the inoculated rats died of plague on March 24 and two on March 25. The epizootic in this godown commenced on March 30 and progressed rapidly, the whole fifteen uninoculated rats being wiped out in a week, as follows:—

March	30	2
„	31	2
April	1	2
„	2	3
„	3	2
„	4	2
„	5	2
				15

The flea count during this epizootic was 11 per rat. No plague rats were eaten. At the end of the experiment, six fresh rats were put in: four of these died of plague.

Godown No. 11. Fifteen uninoculated and five inoculated rats were put in on March 22. All the inoculated rats died of plague by

March 25. The epizootic commenced among the uninoculated rats on March 29, the following series of deaths occurring:—

March	29	1
"	31	3
April	1	2
"	2	2
"	3	2
"	6	1
				<hr/> 11

Five inoculated rats were added on April 7, all of which died of plague by April 11. Another uninoculated rat died of plague on April 16, bringing the total up to 12. A third lot of five inoculated rats was added on April 19, all of which died of plague by April 22. No more uninoculated rats developed plague. The average number of fleas during the experiment was thirty per rat. No plague rats were eaten.

Godown No. 12. Fifteen uninoculated rats were put in on March 22, along with five inoculated rats. Three of the inoculated rats died on March 24. Five more added on April 4 and a third lot of five on April 19: all died of plague. The epizootic entirely failed to light up in this godown, a single uninoculated rat dying of plague on April 10. The average number of fleas per rat was 21. No plague rats were eaten. The fourteen survivors and a similar number of rats from the control godowns were inoculated with 1 c.c. each of a 1 in 5000 emulsion of the spleen of a rat dead of acute plague; none of the former died of plague while of the controls 12 succumbed.

Experiment XII. March 22nd to May 2nd.

Godown	Inoculated rats		Uninoculated rats		Plague rats eaten	Flea count
	Put in	Dead of plague	Put in	Dead of plague		
No. 7	15	15	15	○	0	One flea found on April 25.
No. 8	15	14	15	○	0	Nil.
No. 9	15	14	15	○	0	Four fleas found on April 25.
<hr/>						
No. 10	5	5	15	15	0	Average 11 per rat.
No. 11	15	15	15	12	0	Average 30 per rat.
No. 12	15	13	15	1	0	Average 21 per rat.

SUMMARY AND CONCLUSIONS.

Taking the whole series of twelve experiments, involving twenty-two observations in godowns infested with fleas and the same number done parallel in control godowns, we obtain the following crude figures:—

	Control godowns	Flea godowns
Inoculated rats put in	253	185
„ „ died of plague	217 = 85·7 %	158 = 85·4 %
Uninoculated rats put in	382	381
„ „ died of plague	17 = 4·4 %	163 = 42·8 %
Plague rats eaten	23	20
Fleas present	Few or none	Some or many.

In many cases however—fourteen out of twenty-two—the control godowns did not remain absolutely free from fleas throughout the experiment. Excluding these, and taking only the eight experiments which no flea was ever found in the control godowns, together with the corresponding parallel experiments in flea-infested godowns, the figures come out as follows:—

	Control godowns	Flea godowns
Inoculated rats put in	84	70
„ „ died of plague	73 = 86·9 %	63 = 90·0 %
Uninoculated rats put in	125	125
„ „ died of plague	0	57 = 45·6 %
Plague rats eaten	3	1
Fleas present	None	ca. 24 per rat.

In seven other experiments, very few fleas were present, one or two only being found in several cases after the test examinations had shown no fleas for a month or more. These experiments with their corresponding flea godowns give these figures:—

	Control godowns	Flea godowns
Inoculated rats put in	89	70
„ „ died of plague	78 = 87·6 %	64 = 91·4 %
Uninoculated rats put in	105	105
„ „ died of plague	1 = 1 %	38 = 36·2 %
Plague rats eaten	1	5
Fleas present	ca. 1·5 per godown	ca. 9 per rat.

From these results it is clear *that epidemic infection spreads readily enough from rats inoculated with plague to healthy rats living with them under the conditions of these experiments if a good number of rat fleas are*

present, and that under precisely the same conditions¹, except only that fleas are absent or present in very small numbers, no epidemic occurs. In no case did an epidemic arise in the absence of fleas, though in three cases there was no material spread of infection though fleas were plentiful. Since the number of rats dead of plague which were eaten by their companions was practically identical in the two sets of experiments, it follows that there is no reason for thinking that alimentary infection played any part in the production of these epidemics.

It would involve unnecessary repetition to note in detail the various features of interest in the different experiments. Special attention may however be drawn to experiments I and III. In the former though 9 out of 13 rats dead of plague in the control godown were eaten by their companions, only one of 25 uninoculated rats died of plague, while in the corresponding flea godown 1 of 8 was eaten and 13 of 30 caught plague. In the latter, the control godown remained free from fleas for forty days and no uninoculated rat died of plague; fleas then gained access somehow or other and an epizootic of six deaths in nine days immediately occurred.

The reasons for the variation in the flea prevalence and the intensity of the epidemics at different times will be discussed in a later paper when further experiments now in hand are completed.

The *immunity to plague of the rats left alive* was tested at the end of a number of the experiments. The controls used were fresh Bombay rats, many of which are more or less immune. In each case the godown rats and the controls all had the same dose of an emulsion of plague-rat spleen, and in any one experiment the results are comparable. The different experiments cannot however be compared with one another.

A. From flea-infested godowns:—

Godown rats		Control rats	
Tested	Died	Tested	Died
14	0	30	18
9	0	30	18
4	0	2	1
9	0	23	9
24	0	38 ²	10
14	0	14 ²	12

¹ Except for fleas, the conditions varied only in the number of inoculated rats dying of plague, *i.e.* in the quantity of potential infection presented to the uninoculated rats. But it will be seen that in all cases this was less in the flea than in the control godowns, so that, if anything, the uninoculated rats in the flea godowns had *less* chance of being infected than the controls.

² In these cases (Experiments XI and XII) the control rats were those surviving in the control godowns in which there had been no spread of infection.

B. From control godowns in which there had been some spread of infection :—

Godown rats		Control rats	
Tested	Died	Tested	Died
12	4	20	19
10	8	20	19
9	3	23	9

C. From control godowns which had remained flea-free :—

Godown rats		Control rats	
Tested	Died	Tested	Died
16	15	20	19
16	7	23	9

It is not worth while attempting a numerical expression of the results, but it is clear that *the rats which survived these experimental epidemics were very resistant to plague*. The same thing is shown by the failure (Experiments XI and XII) to restart an epidemic among the survivors by introducing fresh inoculated rats. In all 29 out of 75 rats died after the first dose of inoculated rats; only three of the 46 survivors died after a second dose, and none out of 17 which were tested by a third dose. But it is questionable whether the resistance of the survivors was greater than that of the same animals when the experiment started. Thus the 74 rats from flea-infested godowns, none of which succumbed to subcutaneous inoculation of plague, were the ultimate survivors of 143 wild Bombay rats, 60 of which had died from flea infection in the godowns. In other words, 74 of 143 (52 %) were resistant, or, excluding the rats which died from causes other than plague in the godowns, 74 of 135 (55 %). With doses of the order of those used in making these immunity experiments, it is not improbable that 50 % of fresh Bombay rats would survive (see also vol. VI. pp. 505, 506). As a matter of fact about 45 % of the fresh rats used as controls did not die of plague, so that there is no evidence that there was any acquisition of immunity during the course of the experiments from subminimal infections.

APPENDIX.

The following table shows the distribution of the primary bubo in rats dying of plague contracted in the godowns in Experiments VIII—XII.

Cervical	84
Axillary	4
Inguinal	1
Pelvic	1
No bubo	3
Too much eaten for diagnosis	4
Total					<hr/> 97

It will be remembered that the same preponderance of cervical buboes was found in wild rats naturally infected with plague, in rats infected by transference of fleas and in guinea-pigs infected in the previous godown experiments (see vol. VI. p. 465, vol. VII. pp. 382 ff.).

XXXIV. RESOLVING (CHRONIC) PLAGUE IN RATS.

IN previous numbers of these reports (*Journal of Hygiene*, vol. VI. p. 530 and vol. VII. p. 457) an account has been given of a condition met with in rats to which the name "chronic plague" was applied. Recent observations in Belgaum, Poona and Bombay have thrown additional light on the significance of this condition and have added considerably to our knowledge of the subject. We propose in this paper to give a short account of these observations.

At the outset we may state that we have come to regard the term "chronic plague" as an inappropriate and misleading name for the condition to which we formerly applied it. In a previous paper we pointed out that Kolle was the first to describe what he called "chronic plague" in certain experimental rats, and we referred to the fact that Dimond and others had suggested that plague infection may remain latent or continue to exist for some time in a chronic form among rats (vol. VI. p. 530). We ourselves, when this condition was met with for the first time among the rats in the Punjab village Kasel, were inclined to attach considerable importance to the discovery as affording a possible explanation for the apparent persistence of infection in this village during the quiescent plague period and as furnishing a source from which the seasonable re-appearance of plague in an acute form might have its origin. Subsequent experience, however, caused us to express the opinion (vol. VII. p. 471) "that we had no direct evidence that chronic plague as it occurs in the Punjab villages, possesses any significance in the seasonal recurrence amongst the rats of the infection in an acute form, nor is any evidence available which excludes this possibility."

A much more extensive experience of this condition during the past two years has fully convinced us that the pathological appearances we have described as chronic plague are stages in the process of recovery from the acute disease. For the reasons, therefore, that the

PLATE No. VIII.

Resolving Plague of the Visceral type.

The accompanying plate represents in a semi-diagrammatic manner the more common types of lesions met with in resolving plague of the visceral type. The sketches were made from rats met with during our Belgaum observations and are in each instance an accurate representation of the condition of the spleen. The lesions in each of the six rats illustrated were confirmed as plague by animal experiment.

Fig. 1. (Rat No. 26008.) *a, a, a*, shot-like abscesses each containing a small bead of pus: considerable pressure was requisite to crush these abscesses between two slides owing to the thickness of the abscess wall. *b*, a thinner walled larger abscess, protruding from the surface of the spleen and adherent to the mesentery; this abscess, like the smaller ones, was pearly white in colour. *c*, a greyish yellow necrotic patch, indefinite in outline. *d 1, d 2*, adhesions to mesentery. *st*, stomach. The abscesses contained virulent plague bacilli.

Fig. 2. *a, a*, two hard spherical abscesses from one of which a culture of plague bacilli was obtained.

Fig. 3. (Rat No. 27247.) *a*, a wedge-shaped greyish necrotic mass. *b*, a similar necrotic mass, but irregular in outline. *c*, retroperitoneal abscess situated just above the left suprarenal body. *d*, adhesions. *e*, scar in the spleen adherent to the great omentum. The necrotic patches in the spleen as well as the retroperitoneal abscess contained plague bacilli.

Fig. 4. (Rat No. 23570.) *a*, spherical abscess, hard shot-like containing small bead of pus. *b*, a larger thinner walled abscess closely adherent to the stomach, *st*. The spleen was united to the parietes by dense adhesions, *d*. Both abscesses contained virulent plague bacilli.

Fig. 5. (Rat No. 22706.) *a*, a pearly white shot-like abscess. *b*, a wedge-shaped necrotic mass. A culture of plague bacilli was obtained from the abscess. The spleen was adherent to the parietal peritoneum.

Fig. 6. (Rat No. 25505.) *a, a*, numerous small shot-like abscesses: in addition the spleen contains very many minute necrotic foci. A small portion of this spleen was crushed in sterile normal salt solution, and the resulting emulsion was injected into a bandicoot, the animal died of plague on the fourth day.



RESOLVING PLAGUE IN THE SPLEEN.

FIG. 1. (continued)

term chronic plague does not accurately express the true significance of the condition, and because that term has been associated with theories regarding the reappearance of plague in certain places (theories which in our opinion have little evidence to support them) we propose to substitute the term "resolving plague" for "chronic plague."

In compiling our previous reports on this subject, the material we had at our disposal for the study of this condition was derived from some forty-five rats, all of which were caught in the Punjab villages. For convenience of description we divided the lesions met with into two groups, viz.: (a) peripheral lesions, (b) visceral lesions. Under the former head twenty-eight cases were included; twenty-three of these cases were fully confirmed as plague by animal and cultural tests, while five others, although not confirmed in so exact a way, left little doubt in our minds that they were due to plague. Under the latter head seventeen cases were described, all of which were fully confirmed by cultural or animal tests.

In Poona and Belgaum the condition was found fairly frequently (see below pp. 347—8), and in view of our experience here it was resolved to re-examine the Bombay rats specially for resolving plague. Accordingly in 1909, from January 5th to 29th (*i.e.* just before the onset of plague) 2200 *rattus* caught alive were carefully examined and four examples of resolving plague found. Later on, between May 23rd and August 3rd (*i.e.* immediately at the close of the active plague season) in 6100 *rattus* we found 10 cases and in 3050 *decumanus* as many as 39.

In our present paper therefore we have to deal with a considerably larger mass of material, comprising thirty-six fully confirmed cases of the peripheral type and forty-one fully confirmed cases of the visceral type, together with a very large number of both types which for one reason or another were not fully confirmed, but which, in our opinion, were almost certainly cases of resolving plague; our reasons for holding this opinion will be given later. In this latter category we have placed one hundred and seventy rats with lesions of the visceral type and two hundred and eleven rats with lesions of the peripheral type. The places in which these rats were found are as follows:

Name of place	Peripheral confirmed	Visceral confirmed	Peripheral not confirmed	Visceral not confirmed
Poona	21	17	114	97
Belgaum	8	19	75	58
Bombay	7	5	22	15
Total	36	41	211	170

Our opinion regarding the pathology of this condition may be stated as follows. In a number of papers embodied in these reports, we brought forward much evidence in support of the view that in almost every instance a rat acquires plague infection through punctures made in the skin by infected rat fleas. Plague bacilli having effected an entrance into the body of a rat in this manner pass by way of the lymphatic channels to the nearest lymphatic gland. In this situation a reaction to the bacillary invasion on the part of the tissues of the host is made manifest by the development of a bubo in the majority of instances. On the one hand, the bacilli in their progress towards the blood stream may be arrested in the bubo, or, on the other hand, the bacilli may multiply rapidly and pass on through the gland to the blood stream giving rise to a bacteraemia. In the former case, either of two events may happen: (a) the reactive changes which brought about the arrest of the bacilli in the lymphatic gland may lead to the complete destruction of the bacilli, which is soon followed by the gradual disappearance of the bubo; or (b) the bacilli, although arrested in the lymphatic gland, may continue to survive and thus bring about further tissue changes in the gland resulting in necrosis and pus formation. The bubo is thus converted into an abscess. Such an abscess would furnish us with an example of our peripheral type of resolving plague. The bacilli within the abscess may ultimately be killed, in which case the abscess would be gradually absorbed. A culture from such an abscess would of course be sterile.

The plague bacilli, however, may continue to live within the abscess, which, enlarging by a slow process of necrosis, gradually approaches the skin. Ultimately the abscess may burst through the skin, discharge its contents and finally heal. But, as the outer wall of the abscess approaches the skin, before actual rupture through the skin occurs, secondary infection of the abscess contents by extraneous microorganisms is liable to occur. This secondary infection of the abscess contents is often followed by the disappearance of the plague bacilli within the abscess. If cultures are made from such an abscess other organisms may be isolated from it than the one which primarily caused the abscess. For this reason it may be impossible to prove by cultural or animal tests that a particular abscess was primarily due to plague.

We next consider the cases in which the plague bacilli have not been effectually arrested in the bubo but have gained an entrance to the blood stream producing a bacteraemia. In such case again either of two events may happen: (a) the bacteria may gradually increase

in numbers and so bring about the death of the rat, or (b) reactive changes may take place in the blood resulting in the destruction of the plague bacilli and the agglomeration and isolation of those bacilli which survive in the tissues, especially in the liver and spleen. In this latter case the infected rat may ultimately die in the struggle against the invading bacilli, or if the tissue changes which have limited the bacterial invasion finally lead to complete destruction of the bacilli the rat may survive showing in the process of recovery more or less marked pathological changes in its tissues and organs,—changes which, as the recovery becomes more complete, become less and less evident to the eye.

Considerable light has been thrown on the histology of this struggle on the part of the tissues of a rat against invading plague bacilli in the article "On the pathology of the spleen and liver in spontaneous rat plague with observations on experimental infection" by J. G. Ledingham, published in the *Journal of Hygiene*, vol. VIII. p. 359.

Ledingham divided the cases he examined into two main groups:—

(1) Those in which bacteriaemia of the spleen and liver is at a maximum and has been of recent development.

(2) Those in which bacteriaemia is less prominent or is rapidly disappearing as a result of reactive tissue changes.

It is with this second group that we are now particularly concerned.

Ledingham states that in this group definite abscess formation in the spleen is frequent and is accompanied by extensive reactive changes on the part of the plasma cells. Even with a low magnification large clumps of bacilli may be seen throughout the splenic pulp each surrounded by a zone of karyorrhctic nuclei. Outside of this zone is another of vesicular epithelioid cells and beyond this again a barrier of plasma cells. The condition is thus one of multiple small abscess formation. Very similar appearances are to be found in the liver. The number of these necrotic foci may be very great and their size may vary from a point almost invisible to the naked eye to a well defined and easily recognised abscess. Ledingham remarks that "it can readily be conceived how, provided the animal lives long enough, the reaction of the fixed tissue cells may proceed to complete encapsulation of the abscess areas and so bring about a more or less chronic condition."

The material which Ledingham examined was collected in Bombay by the Commission and was derived from a number of naturally infected acute plague rats.

Ledingham further studied the histological changes in certain experimental rats infected in England and suffering from what he regarded as "chronic" plague.

He describes in particular the lesions found in one of these chronic plague-infected rats. The tissues derived from this rat showed more advanced changes than those detailed above. In this case, he says, referring to the spleen: "In fact the organ was transformed into a veritable plasma cell granuloma with abscesses interspersed here and there. A large clump of degenerated bacilli occupied the centre of each necrotic area and all around were broken down polynuclear cells. Bounding this zone of degenerated cells was a band of epithelioid cells and numerous giant cells of tubercular type. Megakaryocytes also appeared in this zone. Enclosing the whole was a barricade of plasma cells in active division and transition forms were readily demonstrable between these latter cells and the spindle cells from which the granulation zone surrounding the abscess was being developed." The changes in the liver of this rat are particularly interesting: according to Ledingham "A section through one of the subcapsular nodules showed that nothing remained of the original abscess. The nodule consisted solely of spindle cells and fine connective tissue fibres with a boundary zone of actively proliferating plasma cells." It is important to note that the rat presenting these "chronic" lesions was killed so soon after experimental infection with plague as the eleventh day. A reference will be made later to the comparatively transient nature of these so-called "chronic" plague lesions.

It will readily be appreciated that no very hard and fast line can be drawn between the histological changes we have described above which were associated with acute plague in rats and those of this experimentally infected "chronic" plague rat just mentioned. The naked eye pathological appearances in the rats first mentioned led the Commission to regard them as suffering from acute plague although a histological examination by Ledingham showed that many of them were on the road to recovery. The naked eye pathological changes observed in the last mentioned rat however placed it without doubt in the class we are now considering, namely chronic plague rats with visceral lesions or, as we would prefer to say, the class of rats suffering from the visceral type of resolving plague.

To distinguish acute plague-infected rats from rats with resolving plague lesions we were compelled therefore to lay down more or less arbitrary rules. We were guided in this matter chiefly by our former

experience recognising as we did certain well marked pathological lesions to which we had given the name "chronic" plague. These lesions were comparatively easily distinguished by the naked eye from those we associated with the term acute plague. We decided to classify under the term resolving plague only those rats which showed:

(a) A very markedly localised plague lesion readily visible to the naked eye.

(b) The entire absence of those changes which we have described in vol. VII. pp. 526—535 which we associated with acute plague.

(c) The absence of plague bacilli from the body, except in the well defined lesions.

(d) Rats which in our opinion were not likely to die from the plague lesions from which they suffered but were in short recovering from the acute disease¹.

We may now pass on to describe more in detail the naked eye appearances and the general results obtained from an examination of the material we have collected together under the heading resolving plague.

It will not be necessary here to discuss further that form of resolving plague we have classed as the peripheral type in view of the remarks we have made above regarding the pathology of this condition. It will suffice again to reiterate that in a very large number of these cases it is impossible to demonstrate the presence of plague bacilli in these lesions, either because in the process of resolution the plague bacilli have been greatly reduced in numbers, have been completely destroyed, or cannot be cultivated in the presence of organisms which have invaded the lesions secondarily.

Two lines of argument however may be put forward in support of the view that the large majority of these abscesses situated in lymphatic glands are really old plague lesions although the presence of the plague bacillus cannot be demonstrated in them.

First it may be argued from the figures we have collected in Poona and Belgaum, which are given in the annexed tables, that the number of abscesses in lymphatic glands in which plague bacilli were not demonstrated occurred each month with a frequency which closely

¹ The term resolving plague could without doubt be correctly applied to certain other rats which, if a microscopical histological examination had been made, would leave no option but to conclude that they were recovering from acute plague although the evidence might have shown that they had died from the disease. Such rats which had just passed out of the acute phase of the disease have been excluded from our present category.

followed the frequency with which acute plague was found among the rats. There is a distinct correlation between acute plague in rats and these abscesses in lymphatic glands in which the presence of the plague bacillus could not be proved.

Secondly it may be argued that the distribution of the abscesses in the different groups of lymphatic glands corresponds fairly closely with the usual distribution of the buboes in acute plague infected rats.

Thus for example in Belgaum throughout the year 74 rats were found with abscesses in lymphatic glands which were classed as primarily due to plague although the bacillus could not be demonstrated in them. The distribution of these abscesses in the various groups of lymphatic glands was as follows:—

Submaxillary	45 = 61 %
Axillary	7 = 9 %
Inguinal	21 = 28 %
Submaxillary + axill.	1 = 2 %
Total	<u>74</u>

We can compare these figures with the distribution of the buboes in 130 acute plague infected rats caught in Belgaum during the same period. Of the 130 rats, 112 only presented buboes and these were distributed as follows:

Submaxillary	88 = 79 %
Inguinal	10 = 9 %
Axillary	9 = 8 %
Pelvic	3 = 3 %
Submax. + inguinal	1 = 1 %
Axillary + pelvic	1 = 1 %
Total	<u>112</u>

Except then as regards the larger number of abscesses in the inguinal glands among the resolving plague group as compared with the acute plague group, the distribution of the lesions in the two groups is not very different.

Passing on next to consider the visceral type of resolving plague we may remark that abscesses in the spleen are by far the most common manifestation of this form of plague.

The abscesses in the spleen are most often spherical and shot-like. They are usually pearly white in colour and embedded in the substance

of the spleen. They can be shelled out of the splenic tissue without difficulty. In size they vary from that of a millet seed to that of a large pea. Such abscesses have very thick walls and are hard. Considerable pressure is required to rupture one of them between two glass slides. Sometimes the abscesses protrude prominently from the surface of the spleen. Such abscesses usually have thinner walls than those more completely embedded in the splenic tissue.

The abscesses in the spleen may be single or more commonly they are multiple; as many as twenty-four have been counted in one spleen. The spleen which is the seat of the abscesses is generally though not always enlarged; the enlargement is rarely very great. Spleens which have abscesses protruding from their surface in the majority of cases are adherent to the abdominal parietes, great omentum, or mesentery or to all three. The adhesions are always in the neighbourhood of the abscesses and may be long and thin, or the whole spleen may be closely bound down in the midst of dense tough adhesions. Figures 1, 2, 4, and 5 represent semi-diagrammatically some of the appearances met with in rats. In the centre of each of the smaller abscesses there is generally a small bead of pale yellow pus. The pus is often inspissated. Microscopical examination of the pus occasionally reveals the presence of involution forms of plague bacilli; prolonged search is sometimes necessary to demonstrate them: they are rarely very numerous.

Generally the bacilli are seen free in the liquor puris; occasionally, however, they are found phagocytosed within the pus cells. Not infrequently microscopical examination fails to reveal the presence of any organisms.

Cultures have on several occasions demonstrated the presence of the plague bacillus when microscopical examination has yielded a negative result. To obtain an uncontaminated culture from the smaller of these splenic abscesses is frequently a matter of considerable difficulty. On several occasions a culture of the *Bacillus pestis* has been obtained by shelling out a small abscess from the spleen, crushing it between two sterile slides and inoculating a culture tube from the minute head of pus that is adherent to one or other glass slide. It is always advisable when the abscesses are multiple to endeavour to obtain cultures from more than one abscess, as the pus in some of the abscesses is often sterile. A method that has been adopted with successful results for the purpose of demonstrating the presence of *Bacillus pestis* in minute splenic abscesses is as follows:—the abscess is shelled out of the spleen, placed in a sterile capsule and thoroughly crushed and ground up in

normal salt solution. The resulting emulsion is injected subcutaneously into a guinea pig or other susceptible animal.

In addition to this condition of splenic abscesses, rats were not uncommonly met with which had spleens containing necrotic areas or foci rather than true abscesses with purulent contents. These necrotic foci vary much in size, some are very minute but large necrotic masses are also met with. These larger necrosed areas may be irregular in shape but more commonly they are wedge-shaped. They are greyish-yellow in colour. Examples of such necrotic areas are illustrated in figs. 3 and 5. These necrotic areas may be met with alone or in conjunction with splenic abscesses. The abscesses are probably developed in connection with these necrotic foci. Virulent plague bacilli may be isolated from these areas. Smears from the necrotic material occasionally show the *Bacillus pestis* on microscopical examination, usually as involution forms. As in the abscesses, the demonstration of plague bacilli in these necrosed areas is not always easy. They are frequently not seen on microscopical examination of smears made from the lesions. Cultures may yield a growth of the bacillus when it has not been found microscopically. The easiest and perhaps the most successful method of demonstrating the presence of plague bacilli in these lesions is to grind up a small portion of the necrosed area in normal salt solution and inject the resulting emulsion subcutaneously into a susceptible animal. We may here state that the rubbing of the lesions found in visceral resolving plague into the abraded skin of a guinea pig almost invariably fails to infect the animal with plague. This can, we think, be explained by the paucity of the plague bacilli in the majority of these lesions. Our failure to detect "chronic" plague in Bombay, a reference to which has been made in vol. VI. p. 530, may be thus explained, for we then relied on the cutaneous method for the isolation of the bacillus in almost every case. The single instance of chronic plague in Bombay, which we have described in our previous reports, vol. VII. p. 468, was only detected because cultural methods were adopted in addition to the cutaneous method for the isolation of the bacillus.

We have mentioned above that splenic abscesses are frequently adherent to the omentum, mesentery and abdominal parietes by fine fibrous bands. We have observed instances in all stages of progression from abscesses contained within the substances of the spleen adherent to the mesentery by fibrous bands, to abscesses wholly separated from the spleen lying free in the mesentery or great omentum. We have been able to fully confirm the view which was only tentatively put

ward in vol. VII. p. 467 that these mesenteric abscesses originate from lesions which are first developed in the spleen, we may here also state that we have not been able to obtain any evidence in favour of the view that these mesenteric abscesses have originated from an intestinal infection.

At this stage we may put to ourselves the question: What becomes of these plague abscesses in the spleen, mesentery and liver which we have described above? We may at once state that we have not come across any instance which showed that these abscesses had been the source from which an acute plague infection had been re-established in any particular rat. No case was met with showing at the same time abscesses and the presence of plague bacilli free in the blood stream in any numbers. We have not been able to collect any evidence to show that these comparatively quiescent lesions ever pass into the acute form of the disease. On the contrary we have been able to collect some evidence which goes to prove that these abscesses tend to become absorbed and that in a comparatively short time the large majority disappear leaving behind them scar tissue to indicate their previous existence.

In the description we have given above of resolving plague of the visceral type, we have remarked on the frequency with which the splenic lesions are associated with adhesions of the spleen to the abdominal walls and viscera, more commonly to the mesentery and omentum, less commonly to the suprarenal body, kidney, or uterus. These adhesions may persist long after the splenic abscesses have completely disappeared. The only evidence of the previous existence of the abscesses is to be found in scar tissue and localised patches of perisplenitis. The fibrous bands which persist longest are found connected on the one side with the scar tissue and patches of perisplenitis in the spleen, which indicate the seat of the original abscess, and on the other side to the abdominal walls, omentum, suprarenal, kidney or uterus. These fibrous bands are in the majority of cases long, thin and delicate, or they may be thick and fibrous. The spleen may be so closely bound down to the tissues around it that prolonged dissection is necessary to free it from its surroundings. This condition of perisplenitis with adhesions is, however, not generally a permanent one; most cases resolve completely.

We are of opinion that almost every case of splenic adhesions and perisplenitis found in rats which have passed through a plague epizootic has originated in lesions of the visceral type of resolving plague and represents the final stage in the process of resolution of the

abscesses we have described above. Similar conditions may no doubt arise from other infections.

It is, of course, impossible to prove directly that the sterile abscesses and other more advanced lesions, now represented by scar tissue only, were primarily due to a plague infection; it is impossible to isolate the plague bacillus from them. But other arguments may be brought forward to support the view that they were originally caused by plague bacilli. Thus these sterile lesions have been found (1) to resemble closely both in their structure and distribution similar lesions which have been proved to contain plague bacilli; (2) to show a seasonal prevalence which is correlated with acute plague and with similar abscesses from which plague bacilli have been isolated; (3) to be associated in individual rats with peripheral lesions in lymphatic glands pointing to a primary plague infection; (4) the examination of more than 6000 rats (*M. rattus*) caught in Madras city (which for several years has been free from plague) has failed to reveal the presence of abscesses similar to those described above and which were met with in Bombay, Poona and Belgaum Cities especially towards the close of the plague epizootics: nine examples of scars in the spleen have been found.

We have been able to produce resolving plague experimentally in rats by inoculation: details will be published when a further series of experiments are completed.

SUMMARY.

1. The abscesses in the viscera, especially the spleen, and in the peripheral lymphatic glands, some of which contain living plague bacilli, which have been previously described as "chronic plague in rats" are shown to be plague lesions in the process of healing. The name "resolving plague" is more appropriate.

2. These lesions are found most frequently towards the close of, and immediately after, the acute plague epidemic.

3. They have been found frequently in rats in Belgaum and Poona, and a re-examination of Bombay rats shows that they also occur there. We had previously only found them in the Punjab villages.

TABLE I.

showing total number of rats examined each month in Belgaum, among these the number infected with acute plague and the number suffering from resolving plague both of the peripheral and visceral types, showing separately under the latter heads those in which the presence of the plague bacillus was confirmed by cultural or animal tests, and those in which the presence of the plague bacillus was not demonstrated. The last column shows the number of rats with perisplenitis, adhesions and scars in the spleen (recovered plague).

Name of month	Total <i>Mus rattus</i> examined	Resolving Plague										Recovered Plague	
		Acute Plague		Peripheral Type				Visceral Type					
				Confirmed		Unconfirmed		Confirmed		Unconfirmed			
		Number	Per cent.	Number	Per cent.	Number	Per cent.	Number	Per cent.	Number	Per cent.	Number	Per cent.
June 1908	3390	—	—	—	—	1	·02	—	—	1	·02	2	·04
July	4163	8	·19	—	—	4	·09	—	—	—	—	4	·09
August	3785	12	·31	—	—	3	·08	2	·05	5	·13	14	·37
September	3786	16	·44	—	—	9	·23	—	—	5	·13	35	·92
October	3657	43	1·17	1	·02	12	·32	1	·02	9	·24	48	1·31
November	3109	19	·61	2	·06	6	·19	5	·16	5	·16	27	·86
December	2651	23	·86	5	·22	14	·52	7	·26	17	·63	65	2·45
January	1797	6	·33	—	—	10	·55	3	·16	10	·55	94	5·23
February	1732	3	·17	—	—	11	·63	—	—	2	·11	66	3·81
March	1972	—	—	—	—	1	·05	—	—	—	—	34	1·7
April	2302	—	—	—	—	1	·04	1	·04	2	·08	19	·82
May	2457	—	—	—	—	1	·04	—	—	—	—	17	·69
June 1909	2704	—	—	—	—	2	·07	—	—	2	·07	20	·73
Total	37505	130		8		75		19		58		445	

TABLE II.

Showing total number of rats examined each month in Poona and among these the number infected with acute plague and the number suffering from resolving plague both of the peripheral and visceral types, showing separately under the latter heads the rats in which the presence of the plague bacillus was confirmed by cultural and animal tests, and those in which the presence of the plague bacillus was not demonstrated.

Name of Month	Total <i>Mus rattus</i> examined	Resolving Plague									
		Acute Plague		Peripheral Type				Visceral Type			
		Num-ber	Per-cent.	Confirmed		Unconfirmed		Confirmed		Unconfirmed	
				Num-ber	Per-cent.	Num-ber	Per-cent.	Num-ber	Per-cent.	Num-ber	Per-cent.
June 1908	3977	—	—	—	—	1	·03	—	—	2	·05
July	5351	—	—	—	—	4	·08	—	—	—	—
August	5946	—	—	—	—	3	·05	—	—	1	·02
September	6115	10	·16	2	·03	14	·23	2	·03	—	—
October	4913	29	·59	3	·06	7	·14	3	·06	14	·28
November	3814	30	·78	3	·07	7	·18	5	·13	14	·36
December	2923	27	·92	7	·24	33	1·13	3	·1	34	1·13
January	2376	6	·25	4	·16	15	·63	1	·04	13	·54
February	2284	1	·04	2	·09	10	·44	2	·09	8	·35
March	2906	—	—	—	—	13	·44	—	—	5	·17
April	2644	—	—	—	—	1	·04	—	—	4	·15
May	2479	—	—	—	—	3	·12	—	—	2	·08
June 1909	2830	—	—	—	—	3	·14	1	·03	—	—
Total	47558	103		21		114		17		97	

XXXV. ON THE SPREAD OF EPIDEMIC PLAGUE THROUGH DISTRICTS WITH SCATTERED VILLAGES¹.

PART I.

CONTENTS.

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¹ The Committee are indebted to Major Lamb, Director of the Pasteur Institute, Kasauli, for the collection and arrangement of these data which he undertook after he had ceased to be a member of the working Commission in India and had joined the Advisory Committee.

III. Previous plague history of the villages infected in each epidemic.

IV. Previous plague history of villages infected at the beginning of each epidemic.

A. Rohtak District.

(a) Epidemic of 1903—04.

(b) „ „ 1904—05.

(c) „ „ 1905—06.

(d) „ „ 1906—07.

B. Mozuffarnagar District.

(a) Epidemic of 1903—04.

(b) „ „ 1904—05.

(c) „ „ 1905—06.

(d) „ „ 1906—07.

C. Amritsar District.

(a) Epidemic of 1902—03.

(b) „ „ 1903—04.

(c) „ „ 1904—05.

(d) „ „ 1905—06.

(e) „ „ 1906—07.

D. Summary.

V. Later plague history of the villages infected in the course of the milder epidemics.

A. Rohtak District.

(a) Epidemic of 1903—04.

(b) „ „ 1905—06.

B. Mozuffarnagar District.

(a) Epidemic of 1902—03.

(b) „ „ 1905—06.

C. Summary.

VI. Later plague history of villages infected at the end of each epidemic.

A. Rohtak District.

(a) End of epidemic of 1903—04.

(b) „ „ 1904—05.

(c) „ „ 1905—06.

B. Mozuffarnagar District.

(a) End of epidemic of 1902—03.

(b) „ „ 1903—04.

(c) „ „ 1904—05.

(d) „ „ 1905—06.

C. Amritsar District.

D. Summary.

VII. The question whether plague tends to recur in villages in successive epidemics

I. INTRODUCTION.

A. *Scope of observations.*

It is well established that plague, once it has gained a foothold in a place, tends to recur every year at the same season and that the plague season varies in different places. It has been demonstrated that in Bombay City the periods between the epidemics are bridged over by cases of acute plague amongst the rats accompanied by a few cases in man. Knowing, then, the factors which determine the rise and fall of the epizootic amongst the rats, once the infection is present, we have a fairly complete conception of the seasonal prevalence of human plague in a city such as Bombay. In the case, however, of a large province, such as the Punjab, with scattered villages, the question of the annual recrudescence in the several villages is not so simply answered. For, it will be remembered that in the two Punjab villages of Dhand and Kasel, which were under close observation by the Commission for a whole year, no acute plague was found amongst either man or rats in the long interval between the epidemics. While this is so, a certain number of rats were caught alive, which, although apparently in good health, harboured living and virulent plague bacilli in chronic abscesses (see above, p. 335).

It is, however, to be noted, that after a careful study of this condition the Commission came to the conclusion that there was no direct evidence that "resolving" plague, as it occurs in the Punjab villages, possessed any significance in the seasonal recurrence amongst the rats of the infection in an acute form, nor was any evidence available which excluded this possibility. Since the observations were made in the Punjab this condition has been further carefully studied in Bombay, Poona and Belgaum, and, as we shall see elsewhere, the conclusion has been come to that the lesions denote rather a gradual and slow recovery from an acute condition than a definite chronic state. From these facts it is evident that we are still without certain knowledge of the cause of the beginning of plague in successive epidemics in the villages of India. It is this problem to which we now give attention.

On consideration of the problem two solutions seem possible. We shall for brevity designate these two hypotheses (1) recrudescence and (2) importation. Recrudescence implies that in each village each successive epidemic has its origin *in situ* from remnants left over from the previous epidemic no matter how far distant, or in other

words, that dregs of the infection remain over from one epidemic and light up to start the next epidemic. It implies, therefore, that plague bacilli in each village survive the interval between two epidemics. From the work which we have already done it is certain that to fulfil this condition the bacillus must find a habitat in an animal body.

Importation, on the other hand, implies that the great majority of the villages became infected each epidemic by the bacilli being brought into them from without. As the bacillus must survive the non-epidemic season in some way or other, we have to postulate for this hypothesis that the infection in one or more villages or towns bridges over the off-season as scattered cases of acute rat plague and perhaps human plague or in some other way. From the centres or foci where this bridging over takes place the infection would spread out, when the conditions become suitable, exactly as must happen when a district becomes infected for the first time. The villages in which the infection is carried over the off-season might of course vary in different years.

The problem then which lies before us is to *determine the relative importance of recrudescence and importation in the spread of the epidemic through a district with scattered villages.*

We have now to state the methods which were adopted to find a solution for this problem. Three districts in the Punjab and in the United Provinces were selected, for the reasons that they had suffered from several consecutive epidemics of plague and that in the offices of the civil surgeons records of the deaths reported from the villages of the districts were available for analysis. A complete list of the villages in the district was prepared by the clerks in the civil surgeons office, men who knew the districts and who had kept the records. Against each village was shown its population and whether it had at any time been plague-infected or not. If it had at any time reported plague deaths, the following information for each epidemic was entered: the date of the first death, the date of the last death, and the total number of deaths. If the interval between the first and last death was more than three or four months, namely, the normal duration of a single epidemic in a village, the number of deaths month by month was recorded, so that any period of freedom from deaths might not escape notice. These data obtained from the district records have been analysed chiefly in the following directions.

First, maps have been prepared showing the infected villages month by month for several years from the first introduction of plague into

the district. In these maps we are able to trace the spread of each epidemic and to compare the later epidemics with the first one, which we know must have spread by importation. The rise of each epidemic can also be graphically followed and can be correlated with the decline of the previous epidemic. The maps of Rohtak district are reproduced.

Secondly, the previous plague history of the villages infected in each epidemic has been determined with the object of obtaining data regarding the relative proportion of those which had never been infected before, of those which had been infected during the previous epidemic and of those which had escaped for either one or more epidemics.

Thirdly, in those villages in which the disease appeared first in each epidemic the previous history as regards plague infection, especially in the epidemic just passed, has been traced.

Fourthly, in some districts only a small number of villages were infected in one or more of the epidemic years. We have traced the fate of all these villages in the following year in which the epidemic was widespread and involved a very large number of the villages of the district.

Fifthly, we have followed in detail the future history as regards plague infection of villages infected at the end of each epidemic, so as to ascertain in what proportion of them deaths were reported early in the next epidemic.

Finally, we have investigated the question whether plague tends to recur in villages in successive epidemics, and have also analysed the relation of the size of a village to its liability to become infected.

In considering the results obtained in this way, it is necessary to bear in mind the limited accuracy of the records. In particular they tell us nothing of rat plague which is not accompanied by obvious human plague. The fact for example that a village has not returned a plague death is not an absolute proof that there has not been a small rat epidemic, accompanied perhaps by a few mild human cases. It is therefore possible that the first year of infection of any village is recorded as being later than it really was. It is, however, impossible to estimate the weight of these possibilities. Considering the intimate domestic relationship of rats and human beings in the Punjab villages, it seems to us unlikely that rat plague could often exist without being indicated by human plague; where this is known to have occurred, the conditions have been very different. In any case, we have no choice but to deal with the data as they stand: we have little doubt that they give a substantially accurate picture of the incidence of plague infection in the villages concerned.

B. *Districts in which observations were made.*

(1) *Rohtak*. Rohtak is a small district in the south of the Punjab. It is about 64 miles long by 43 miles broad and has an area of about 2800 square miles. There are no natural boundaries such as rivers, etc., so that there is free intercourse on all sides. The total population is about 620,822 which gives a density of 0·3 per acre.

There are 499 municipal towns and villages, none of which, however, are very large. There are only three municipal towns, namely, Rohtak (pop. 20,024) and Beri (pop. 9723) in the Rohtak subdivision, and Jhaggar (pop. 12,227) in the subdivision of the same name. The position of these towns is shown on the maps. All other settlements are villages with a population of less than 8000. The average population per village is 1244.

The district is divided for administrative purposes into four subdivisions or tehsils; Gohana in the north, Jhaggar in the south, and two in the centre, namely Rohtak to the west and Sampla to the east. A few details concerning the distribution of the population in the different tehsils are given in Table I. The point in this table to which we would especially draw attention is the relatively small average population of the villages in the Jhaggar tehsil. While the total population of this tehsil is the smallest of all, it is divided up amongst the greatest number of villages, so that the average population per village is from $\frac{1}{2}$ to $\frac{1}{3}$ that of the other tehsils.

Plague was introduced into the Rohtak district in the winter of 1903—04, the first deaths being reported in the Rohtak tehsil in November 1903. Up to July 1907 there were four epidemics, two slight ones alternating with two severe ones (vide Table II). Of the 499 villages in the district 145 were not infected in any of the epidemics, leaving 354 which returned deaths in one or more of the epidemics.

The seasonal prevalence of the epidemics was well marked (Table III). The deaths began to increase each year in the autumn (September—October) or early winter (November—January). The increase continued until a maximum was reached in April or May, after which there was a sudden fall in June. During July and August only a few villages returned deaths or sometimes none at all.

(2) *Mozuffarnagar* is a district in the north of the United Provinces, not far from the southern boundary of the Punjab. It is bounded on the west by the river Jumna, while all along the east side runs

the sacred Ganges. On the north and south there are no natural boundaries. The total population of the district is about 917,896. There are 973 inhabited municipal towns and villages, so that the average population per village is 944. There are only three towns with a population of more than 10,000, namely Mozuffarnagar (23,444), Kairana (19,304), and Kandla (11,563). All the other villages have less than 9000 inhabitants each.

The division of the district into thanas has been adopted for our present purpose. There are 18 of these subdivisions in the district.

Plague was first introduced into the Mozuffarnagar district in January 1902. Up to the summer of that year only four deaths were returned, so that it is improbable that there was any indigenous infection in that season.

In November 1902 the epidemic began in earnest and from then up to 1907 there have been five epidemics, the data referring to which are given on Table IV.

Of the 973 villages in the district, 334 have been at no time infected leaving 639 which have reported deaths in one or more of the epidemics. The seasonal prevalence of the epidemics has been well marked. The deaths began to increase each year in the autumn (September—October) or early winter (November—January). The maximum was reached in April, after which there was a fall until by the end of June the epidemics had practically ceased (Table V).

(3) *Amritsar*. Amritsar is a moderate sized district in the centre of the Punjab lying a short distance from the foot of the Himalayas. It has an area of 1601 square miles, the total population being 1,039,620 which gives a density of about 1 per acre.

Along the south-east limits of the district runs the Beas river, while on the north-west the Ravi separates it from the Sialkot district. There are no natural boundaries along the other two sides of the district, which is roughly square in shape. The main line of railway runs practically from east to west and the district is further intersected by many canals, the general direction of which is from north-east to south-west. There are four municipal towns and 1058 inhabited villages. Of the former Amritsar, the chief town of the district, is by far the largest, its population being 162,429. The three other municipal towns Tarn-Tarn, Majitha and Jandiala have a population between 4428 and 7877. Only very few of the villages have more than 4000 inhabitants. The average population per village, including the municipal towns, is 978. The district is divided into three tehsils,

namely Ajnala in the north, Amritsar in the centre, and Tarn-Tarn in the south.

Plague deaths were first reported from the Amritsar district in February 1902. This epidemic, beginning late in the plague season, had only affected 62 villages when the hot weather limited its further spread. Succeeding this mild outbreak there have been five more or less severe epidemics (Table VI), the mildest being that of 1905—06 in the course of which about a quarter of the villages in the district became infected. The other four epidemics have all been severe, in each instance about half or more of the villages returning plague deaths. There were, therefore, three consecutive seasons, 1902—03, 1903—04, and 1904—05, during which the district was badly infected, a condition of affairs somewhat different to that which has been described as occurring in the Rohtak district, in which two mild epidemics alternated with two severe ones.

Of the 1062 towns and villages in the district 907 have in the course of one or more of the six epidemics returned plague deaths, leaving 155 which have not at any time been infected.

As was observed in the other two districts the seasonal prevalence had been well marked (Table VII). Each epidemic, beginning in the autumn or early winter, has gradually gained strength until the height was reached in April or May. After May the decline has been rapid, so that by the end of July very few villages remained infected; that is to say, returned plague deaths. Finally, it is important to note that since the disease was first introduced into the district in February 1902 until the end of July 1907, there have only been two months, namely, September and October 1903, during which one or more villages did not report plague deaths. In fact, this large area has at no time even in the off-season been free from human plague and as a corollary acute rat plague must always have been present in the district.

Analysis of the data obtained.

II. METHOD OF SPREAD OF THE EPIDEMICS IN EACH DISTRICT AS JUDGED FROM THE MAPS.

The maps, as we have stated, were prepared from the records of plague deaths which were available in the district plague offices. It is to be noted that the criterion of infection is the occurrence of a death

or deaths from plague so that when a village is spoken of as being infected it means that one or more deaths from plague have been reported among the population.

A. *Rohtak District.*

Maps I—XLVIII¹.

(a) *Epidemic of 1903—04.* Deaths began to be returned in November 1903 from two separate villages in the extreme west of Rohtak tehsil and from Beri town situated to the south of the district.

In January 1904 the pattern suggests a slight spread out from Beri and there appears a fresh infection in the extreme south of the Jhaggar tehsil. The maps of the following months show a well marked spreading out from the Beri centre and also an increase of infected villages round about those which first returned deaths in the west and south of the district. The town of Rohtak, the largest in the district, did not become infected till late in the epidemic, namely in April, and there was hardly any spread to the northern portion of the district, the Gohana tehsil remaining practically free. In June the epidemic subsided and in July only one village, to the west of Beri, still reported deaths.

It is important to keep in mind the pattern of these maps. The villages were all infected for the first time, so that the origin of the infection in each case was presumably due to importation. The pattern as we have seen suggests a spreading out as the epidemic progresses from two if not from three foci, into which the disease was introduced at the beginning of the epidemic.

(b) *Epidemic of 1904—05.* This epidemic began in August 1904, that is to say, there was no month between it and the end of the previous epidemic in which plague deaths were not reported from the district. In August two villages returned deaths, one in the Rohtak tehsil quite close to Beri and the other in the south of Jhaggar. It will be remembered that in 1903—04 both these localities had been infected. Further, one of the villages, namely, that in Rohtak, had itself been infected as late as May: the other village had not previously reported deaths.

In September there appears to be a slight spread out from the Beri centre and a new centre crops up in the Gohana tehsil. This latter

¹ In the months for which no maps are given there was no plague in the district. The circles represent epidemics with less than five plague deaths.

village, although not itself previously infected, is situated next to the only village in this tehsil which was infected in the previous epidemic, and in which deaths took place as late as June. It is also worthy of note that the three villages in the neighbourhood of the Beri centre had all been infected late in the last epidemic, two as late as June and the third one up to May.

The October map shows a slight increase of the number of infected villages in the neighbourhood of Beri. Rohtak town is now also infected.

In November and December more infected villages appear around Beri and also in the neighbourhood of the centres established in August in the south of Jhaggar tehsil and in September in the north in Gohana.

It is evident, therefore, that by the end of December the infection had become fairly widespread. The subsequent maps show a gradual thickening of infected villages all over the district, in the January map the spread being traceable from the centres already established, especially around Rohtak town. In June the epidemic markedly subsided, the villages then infected being chiefly situated in the north-eastern portion of the district. In July only a single village returned deaths.

(c) *Epidemic of 1905—06.* During August and September 1905 the district was apparently free from plague as no villages returned any deaths.

In October two villages showed infection, one in the north of Gohana tehsil and the other in the south-east of Sampla. The former had returned deaths as late as June 1905 and the latter a few deaths in May 1905. Further, in June both were surrounded by infected villages.

In November and December there is little change, only one infected village in the north of Gohana being added in December. This village was also infected the previous epidemic as late as June. From now onwards the main feature of the maps is the gradual increase of infected villages in the north of Gohana, round about the villages which first returned deaths. A very few scattered villages, chiefly in Rohtak tehsil, become infected, but there is no appearance of a spread out from any centre except from the one in the north of Gohana. Here the infection lingered up to June, in which month four villages in this tehsil were still returning deaths.

In July no deaths were reported from anywhere in the district.

It is to be remembered that the epidemic of 1905—06 was a slight one, only 30 villages being reported infected.

(d) *Epidemic of 1906—07.* In August 1906 a single village began to return plague deaths. It was situated in the north of the Rohtak tehsil, but only a few miles to the south of the four villages which had reported deaths in June 1906. Further this village had itself been infected the previous epidemic, the last death being reported on the 27th May.

In September three more villages were returned infected: two in Gohana tehsil to the north of the village infected in August, and the third in the extreme east of the Sampla tehsil. Of these three villages only one of them had been infected in 1905—06, the last death taking place on 10th June, 1906.

In October again three new villages reported cases, two in Gohana and one in Sampla tehsil. Only one of these villages had been infected in the previous epidemic.

During the next two months a few more villages in the northern portion of the district became infected making 11 villages which up to the end of 1906 returned deaths. It is important to note that of these 11 villages only four had been infected in the previous epidemic, the others having been apparently free from plague in every instance for at least 15 months. Further, up to the end of December, the infection was entirely confined to the north and east, especially the north, portions of the district.

The remaining maps of this epidemic show a gradual thickening of the infected villages in the north-east with a later incursion into the centre and over the centre into the west. Rohtak tehsil in the west is infected late, namely March, April and May, while Jhaggar in the south is hardly infected at all. In this tehsil up to the end of March only four villages had reported deaths, not one of which had been infected in the previous epidemic. The pattern in short suggests a spread out from the centres established early in the north and east.

B. Mozuffarnagar District.

The first plague death in the district was reported from Mozuffarnagar town in January 1902. This was probably an imported case as nothing further happened.

In April of the same year a village, Biralsi, in Charthawal thana reported three deaths. As there was no further sign of infection in this place till December 1904, these also were probably not indigenous cases.

No more deaths were returned until November 1902, when the first epidemic began.

(a) *Epidemic of 1902—03.* This epidemic was a small one, only 25 villages becoming infected. It was entirely confined to the south-east half of the district suggesting that the invasion had taken place from this direction. It is interesting to note in passing that the railway passes from south to north through this portion of the district.

With the exception of the thana of Budhana, in which several villages close together returned deaths, the patterns of the maps do not suggest a spread out from any definite centres, the villages which became infected being all widely separated from one another. We would especially draw attention to this springing up of the infection in villages at a distance from one another. This fact is of the greatest importance as, it is to be remembered, we are now dealing with the first epidemic in the district when every village presumably owed its infection to importation.

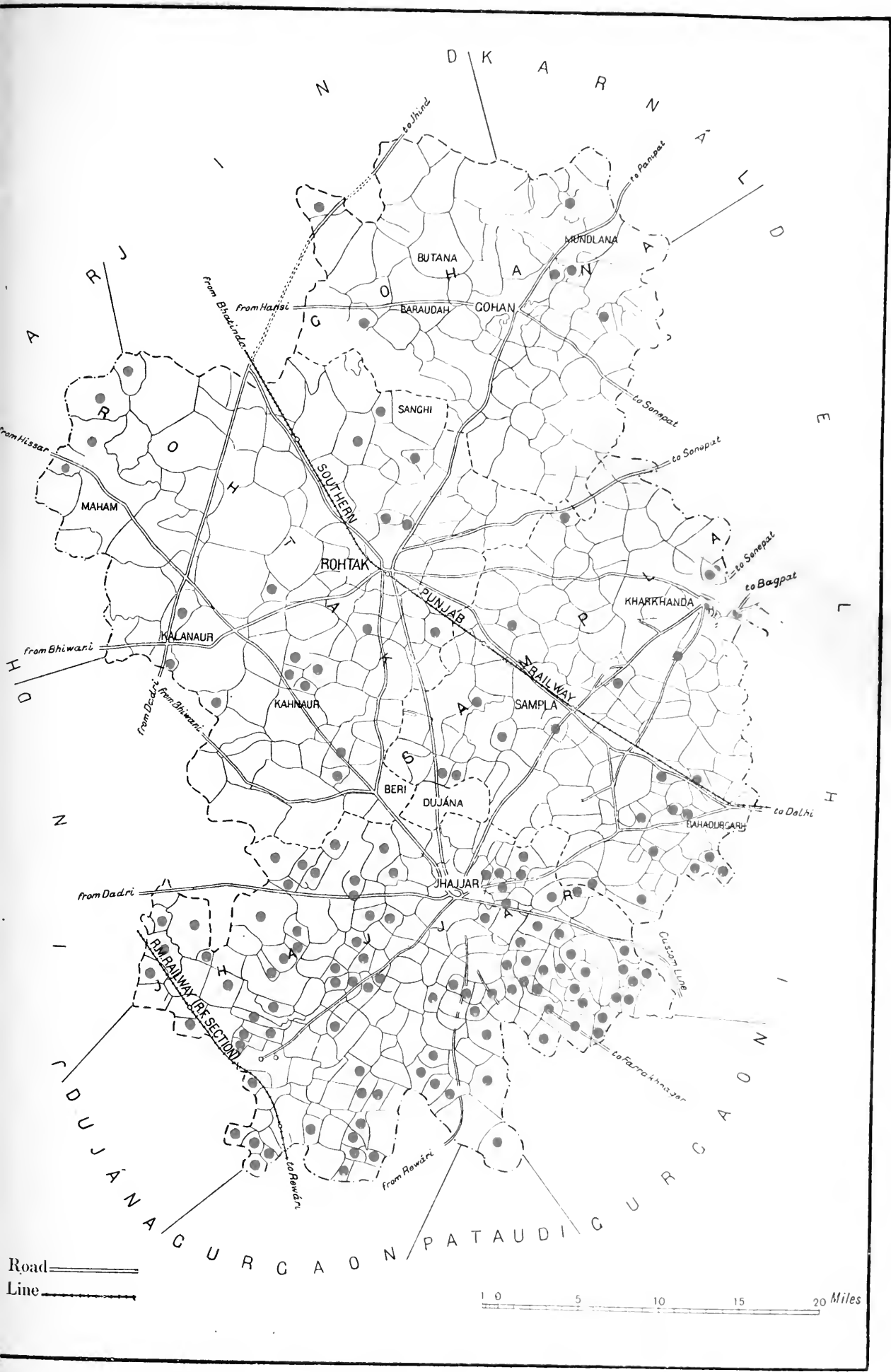
(b) *Epidemic of 1903—04.* The epidemic of 1902—03 came to an end in the month of June 1903. During July and August of this year no villages reported plague deaths.

The epidemic of 1903—04 began in two towns, both of which had been infected late in the previous outbreak and showed an interval of only three months free from deaths.

The patterns of the maps suggest a spread out from these centres and at the same time the development of fresh infections further afield, which villages again become the centres of spread. It is to be noted that the great majority of the villages which reported deaths in this epidemic had never been infected before. Thus of 130 villages which returned cases, only nine of them had been infected in 1902—03, so that in the case of at least 121 (93 %) villages the origin of the outbreak may be attributed to importation.

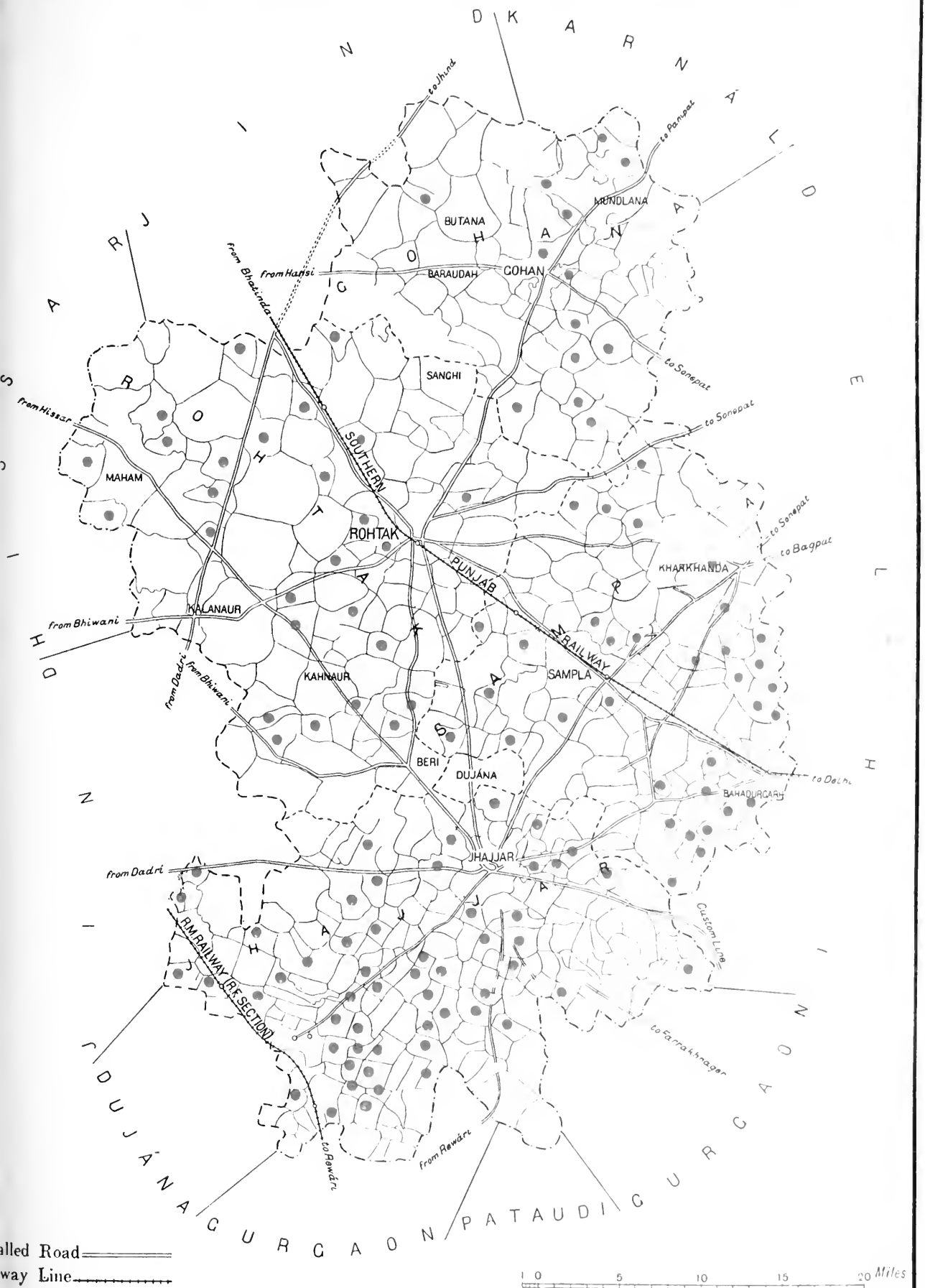
(c) *Epidemic of 1904—05.* Taking this epidemic to have begun in July 1904, there was no interval of freedom between it and the previous outbreak.

Tijalhera, a village in Purkazi thana, became infected in April 1904 and reported deaths right through the off-season till 10th September, 1904; Bebra in Bhopa thana became infected in May 1904 and continued without interruption to return deaths until 7th October, 1904. In July, as well as these two villages, two others close together in Charthawal thana each reported two deaths. They had never been infected before.



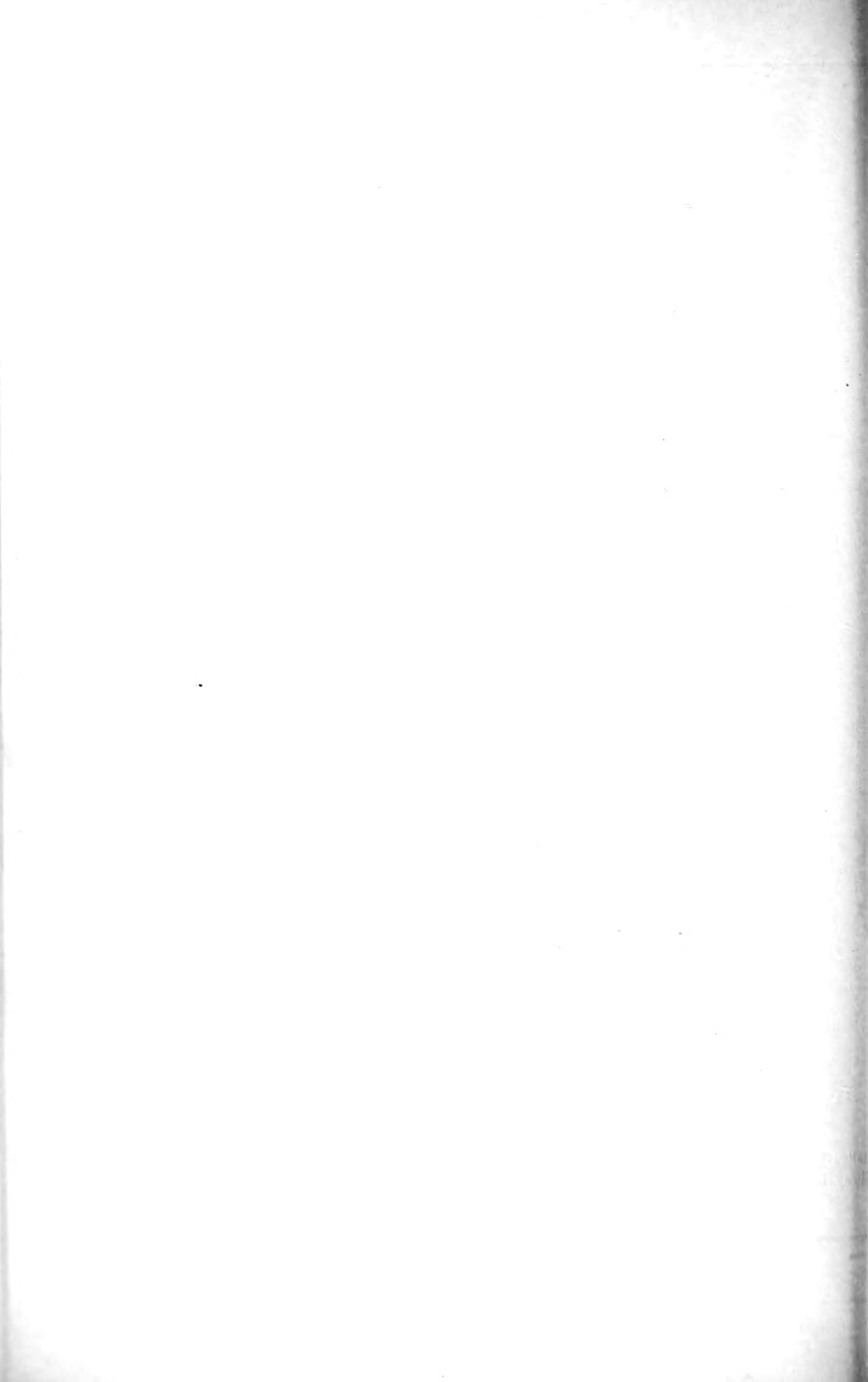
ROHTAK

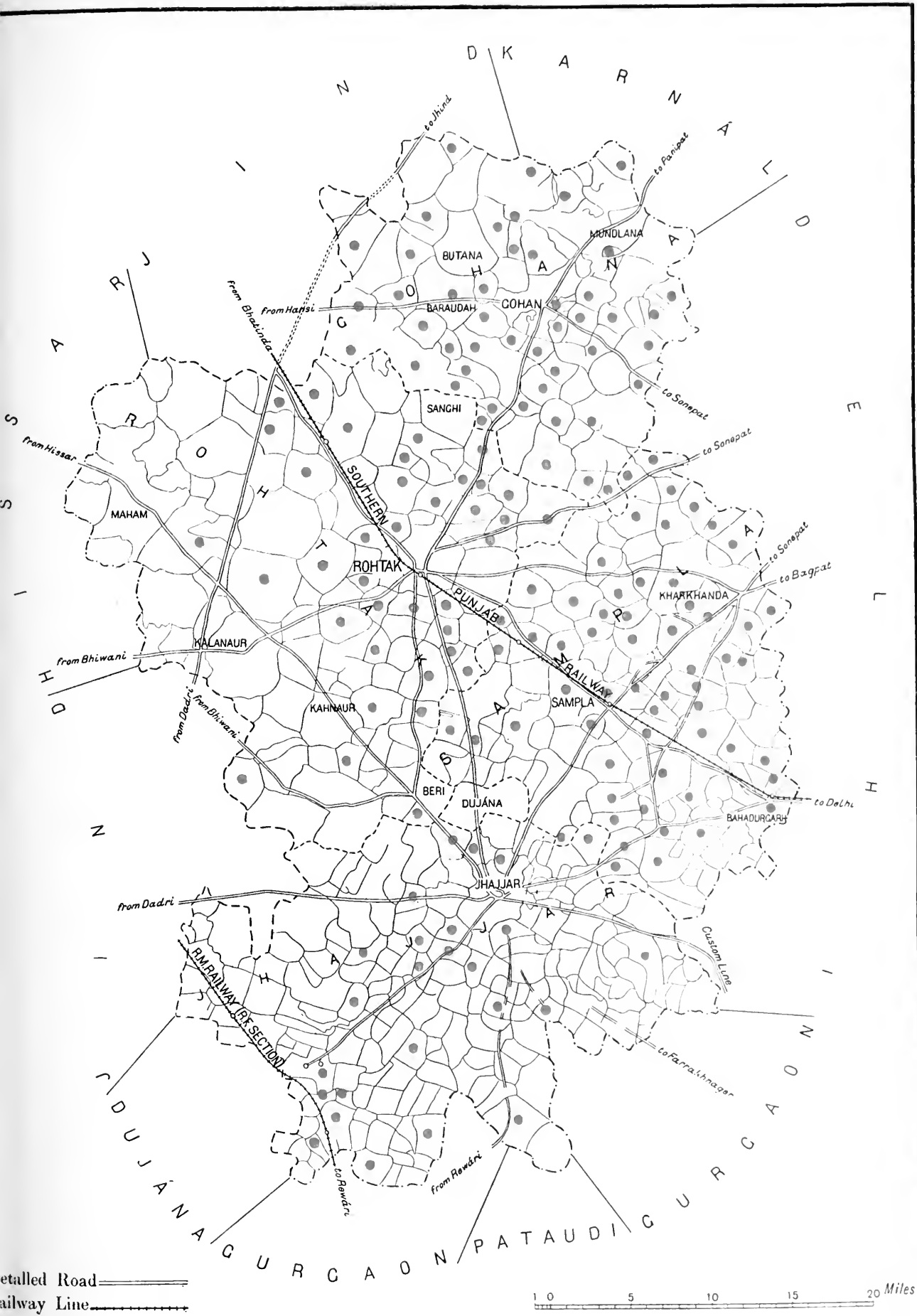
Villages not infected in any of the epidemics



ROHTAK

Villages infected in one epidemic

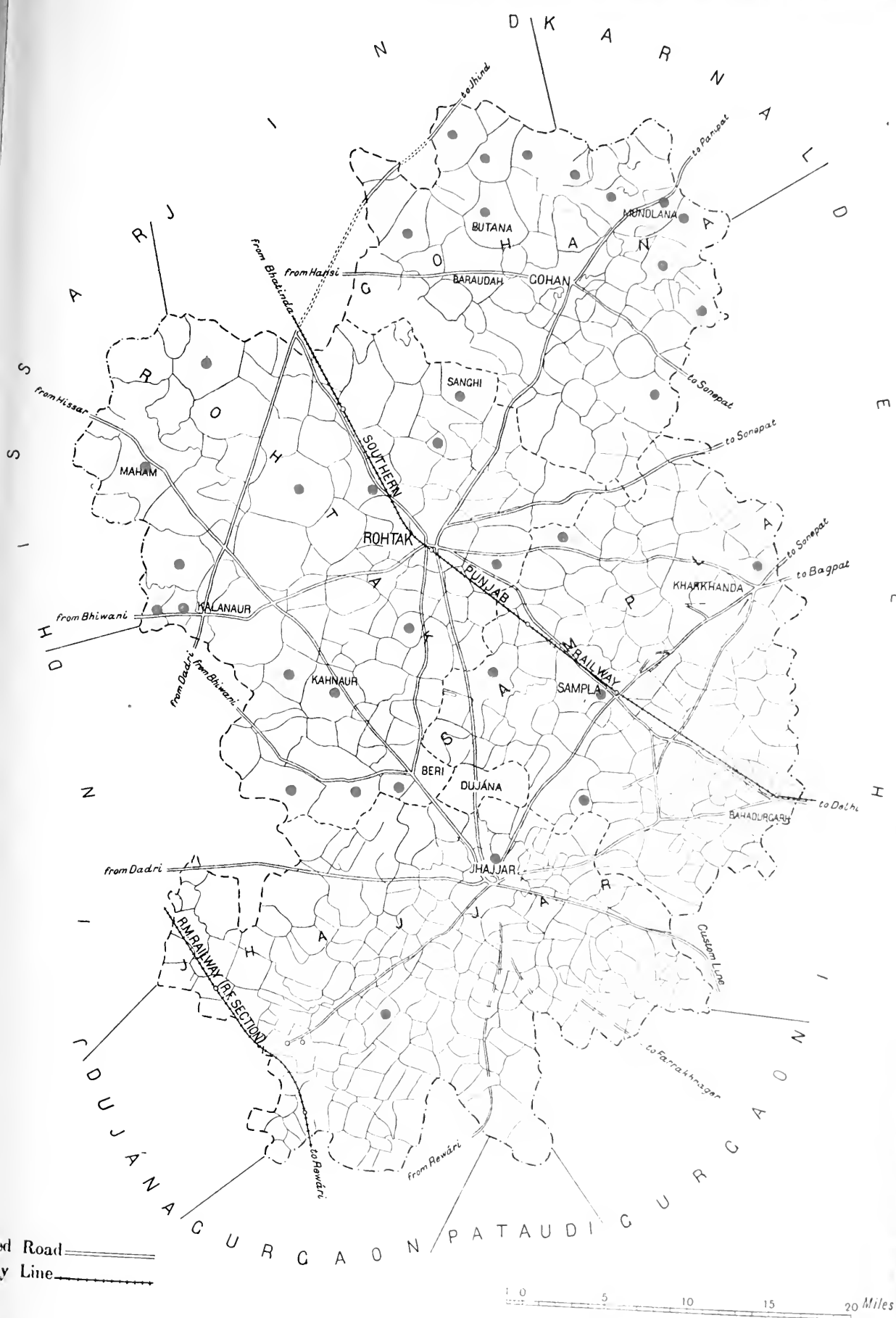




ROHTAK

Villages infected in any two epidemics

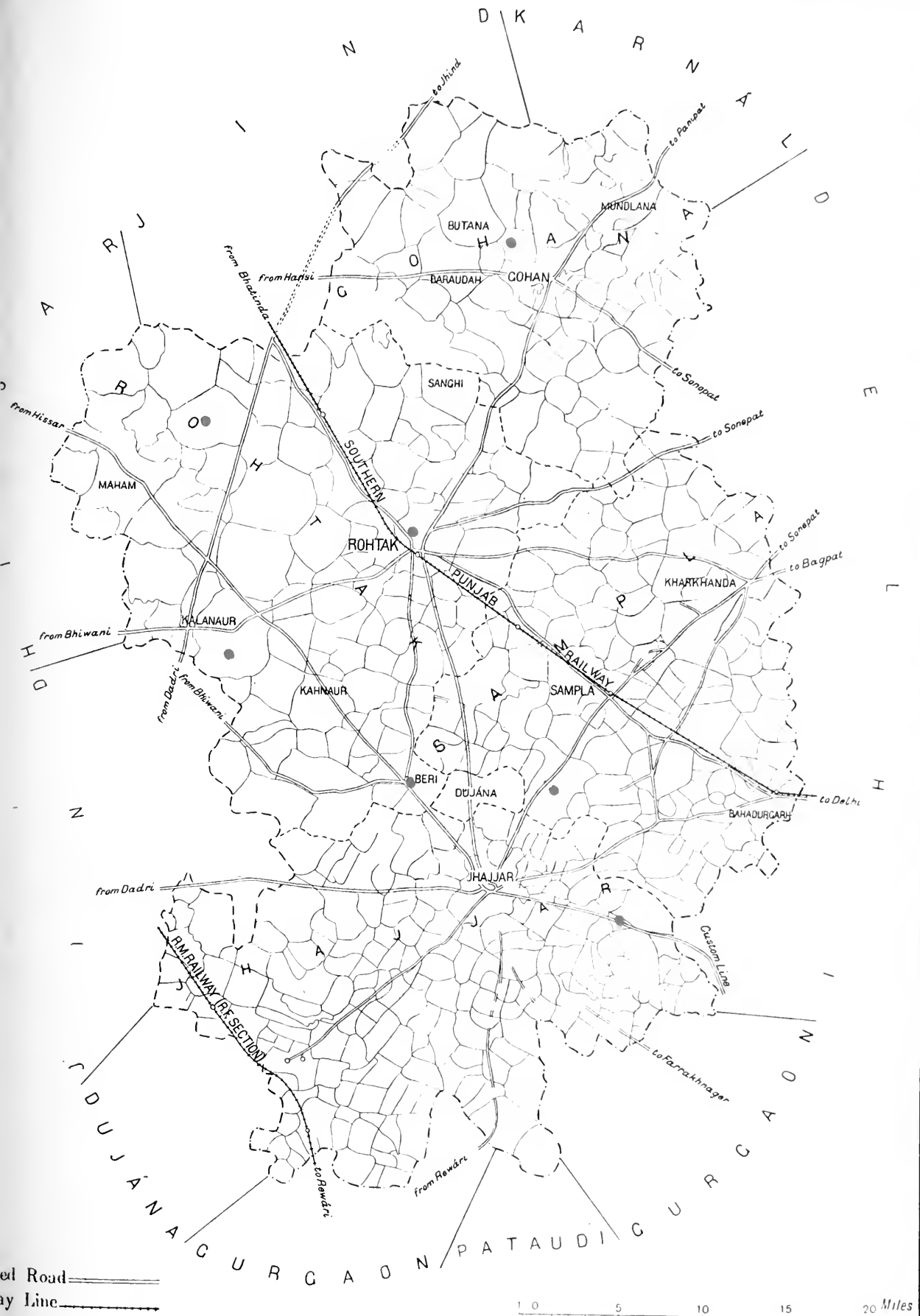




ROHTAK

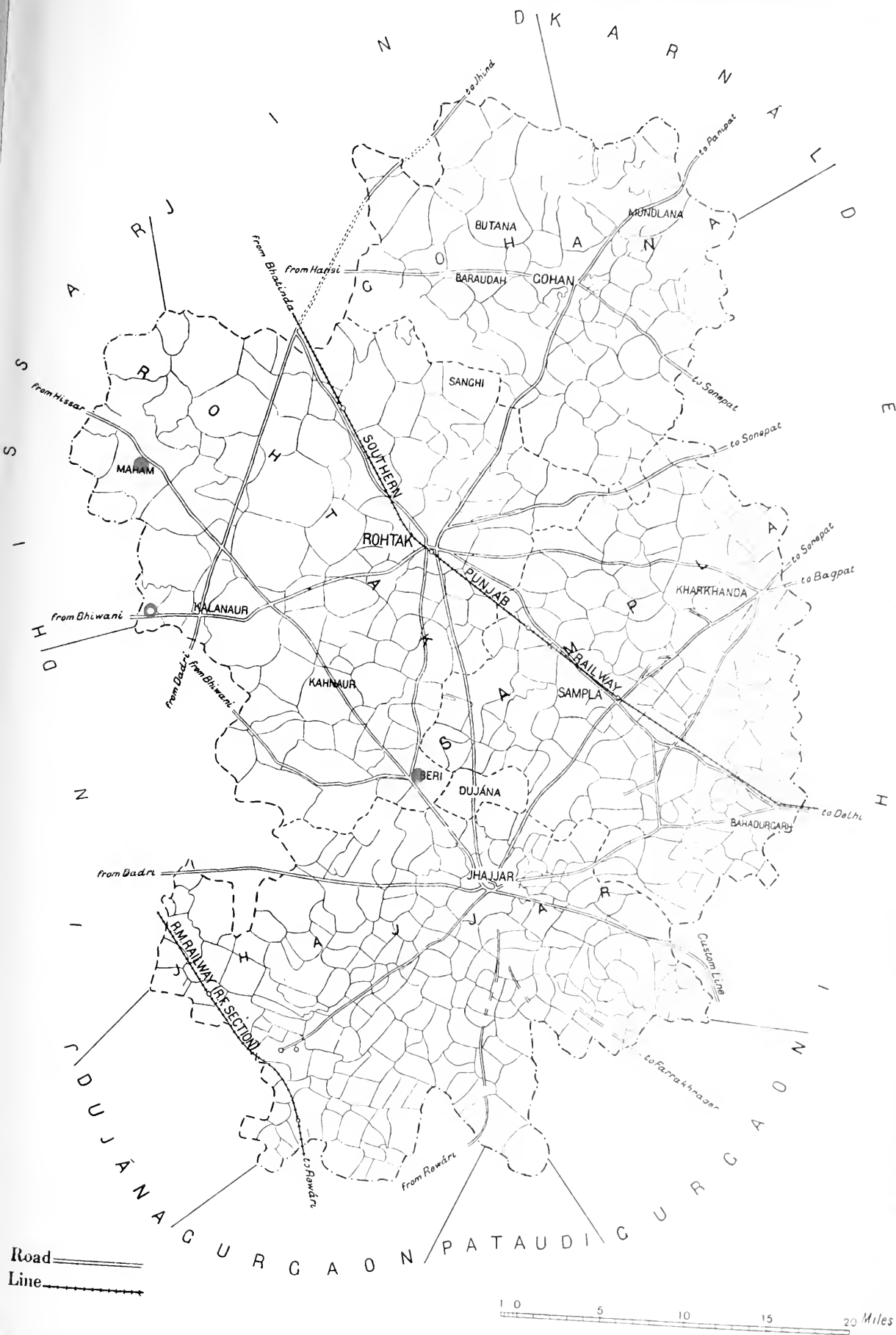
Villages infected in any three epidemics





ROHTAK

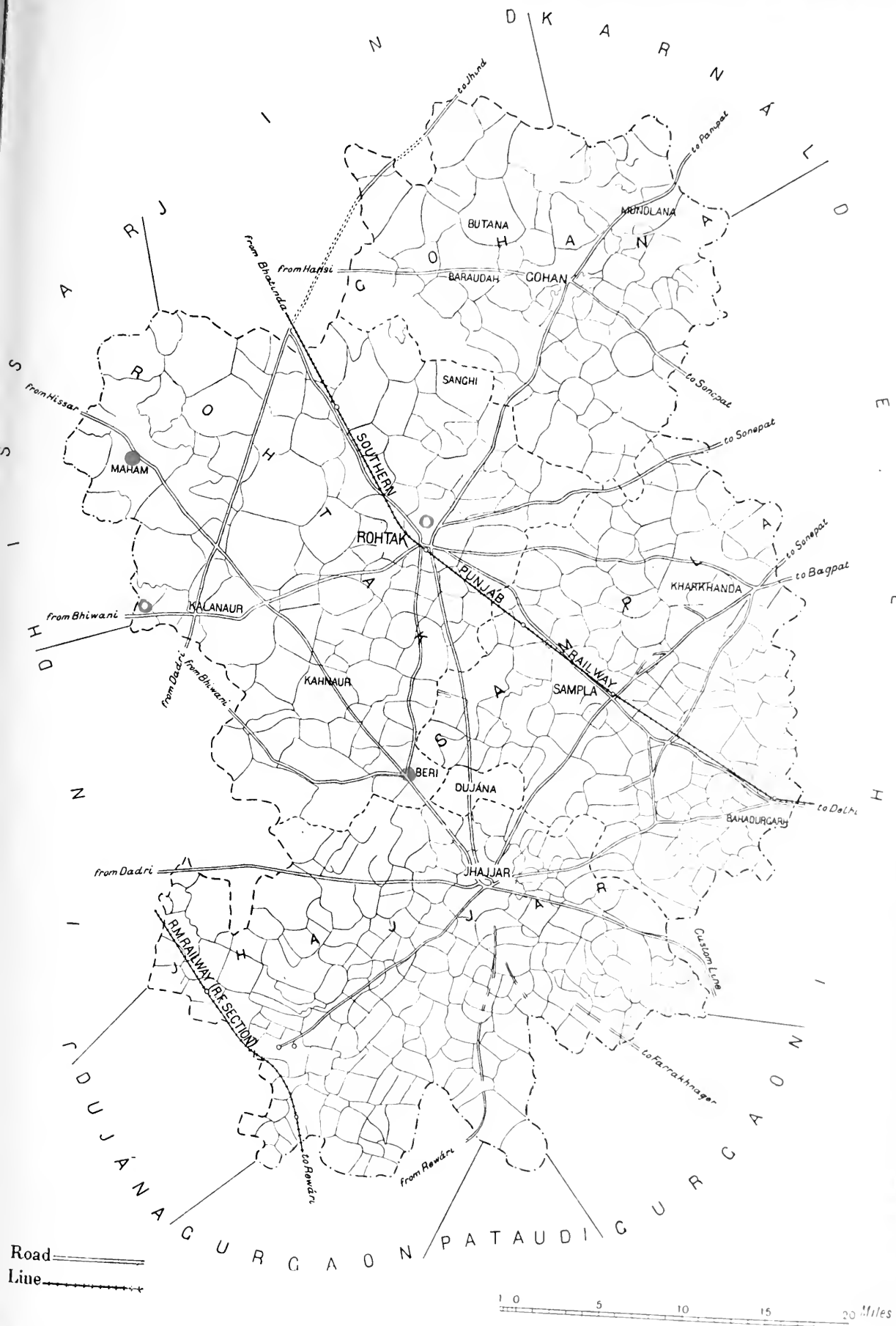




ROHTAK

November

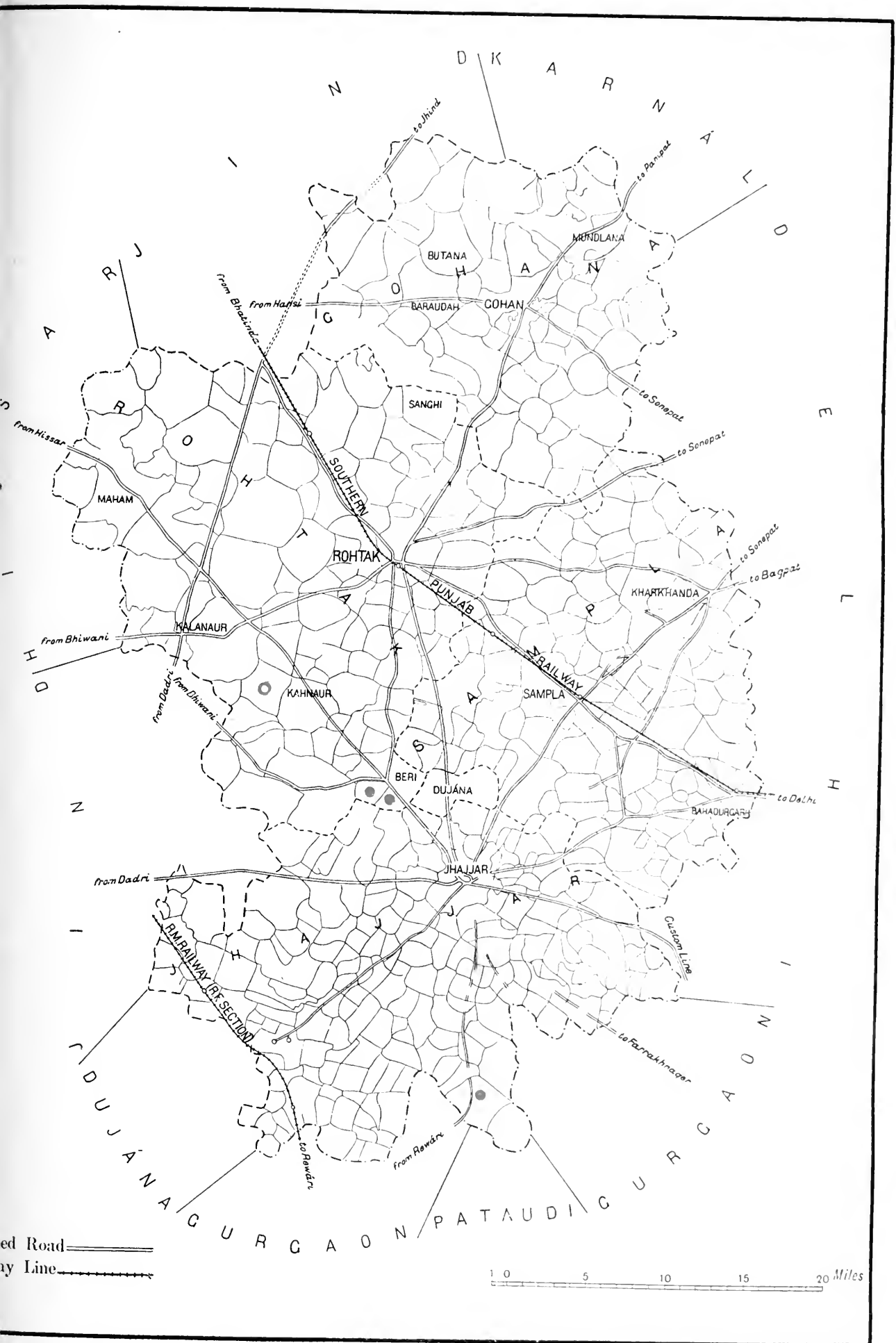


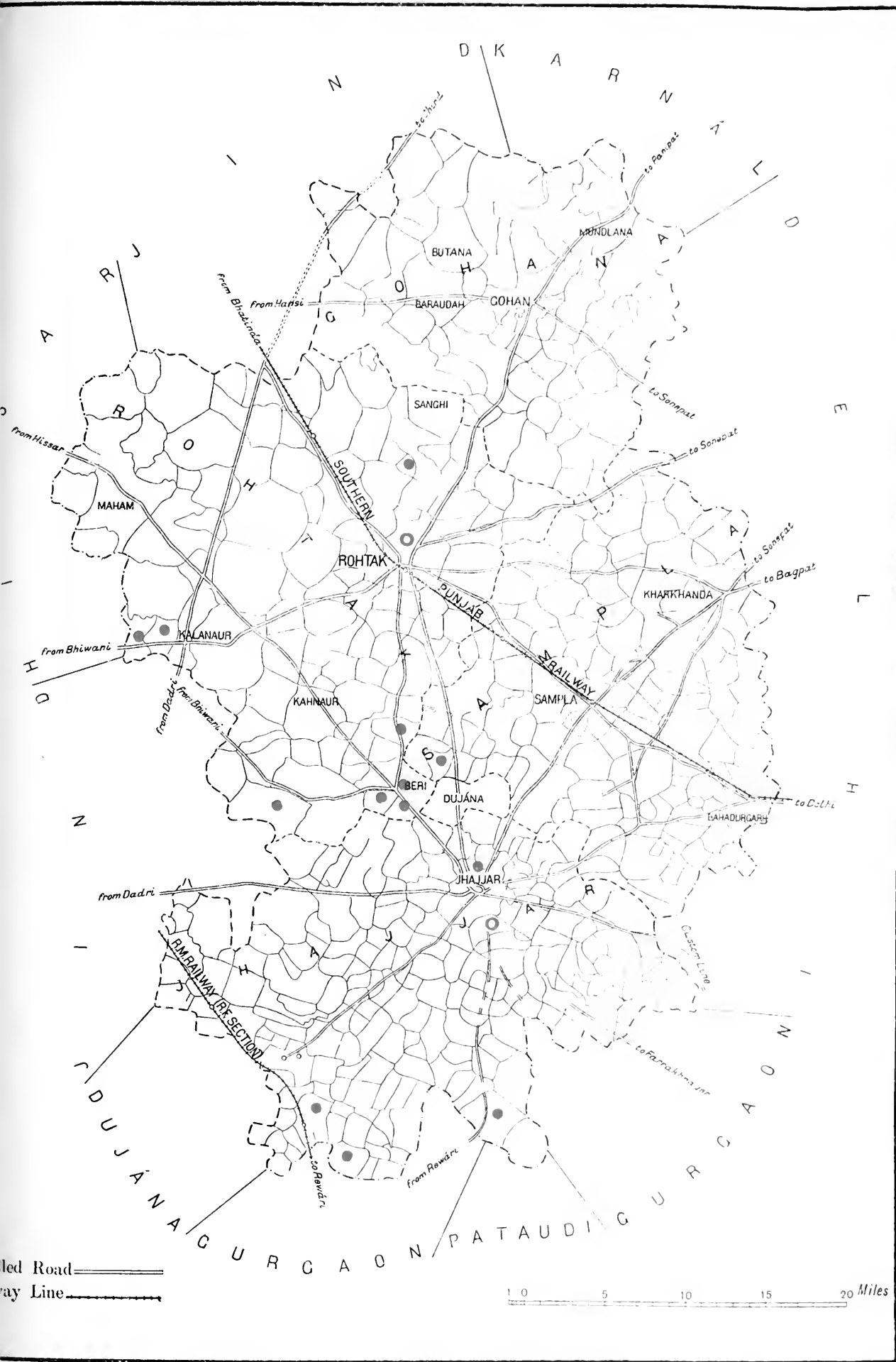


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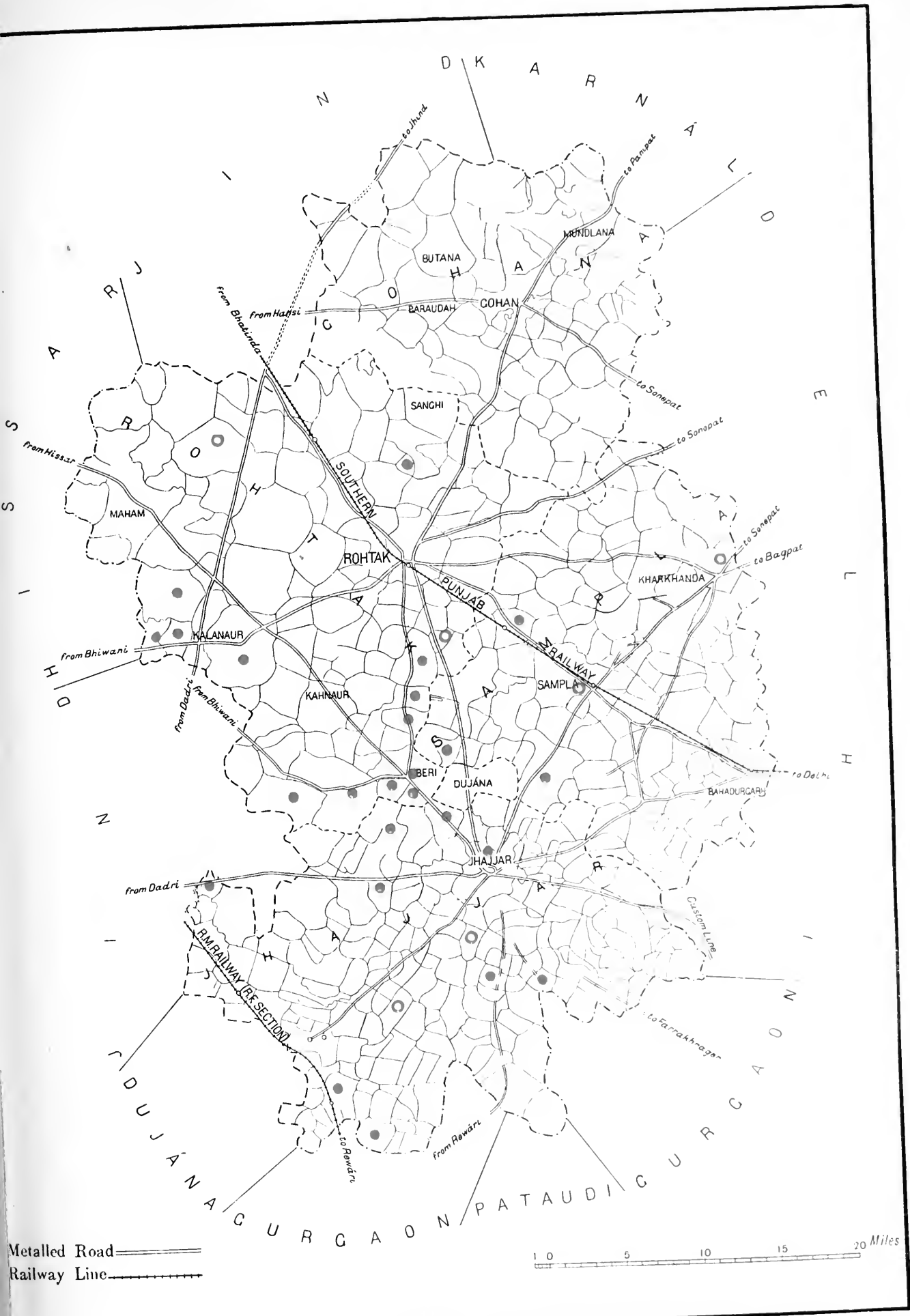
December 1905





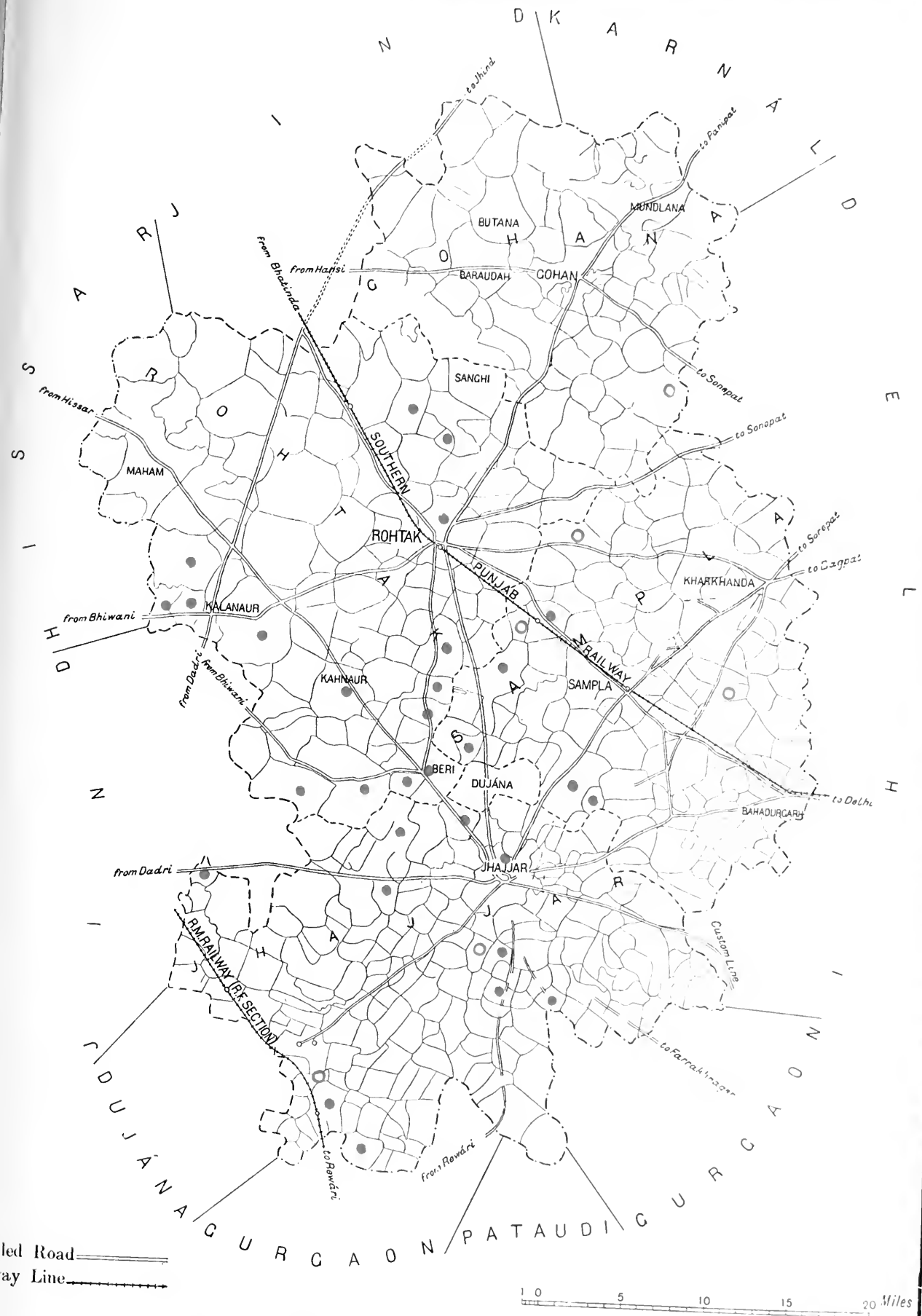






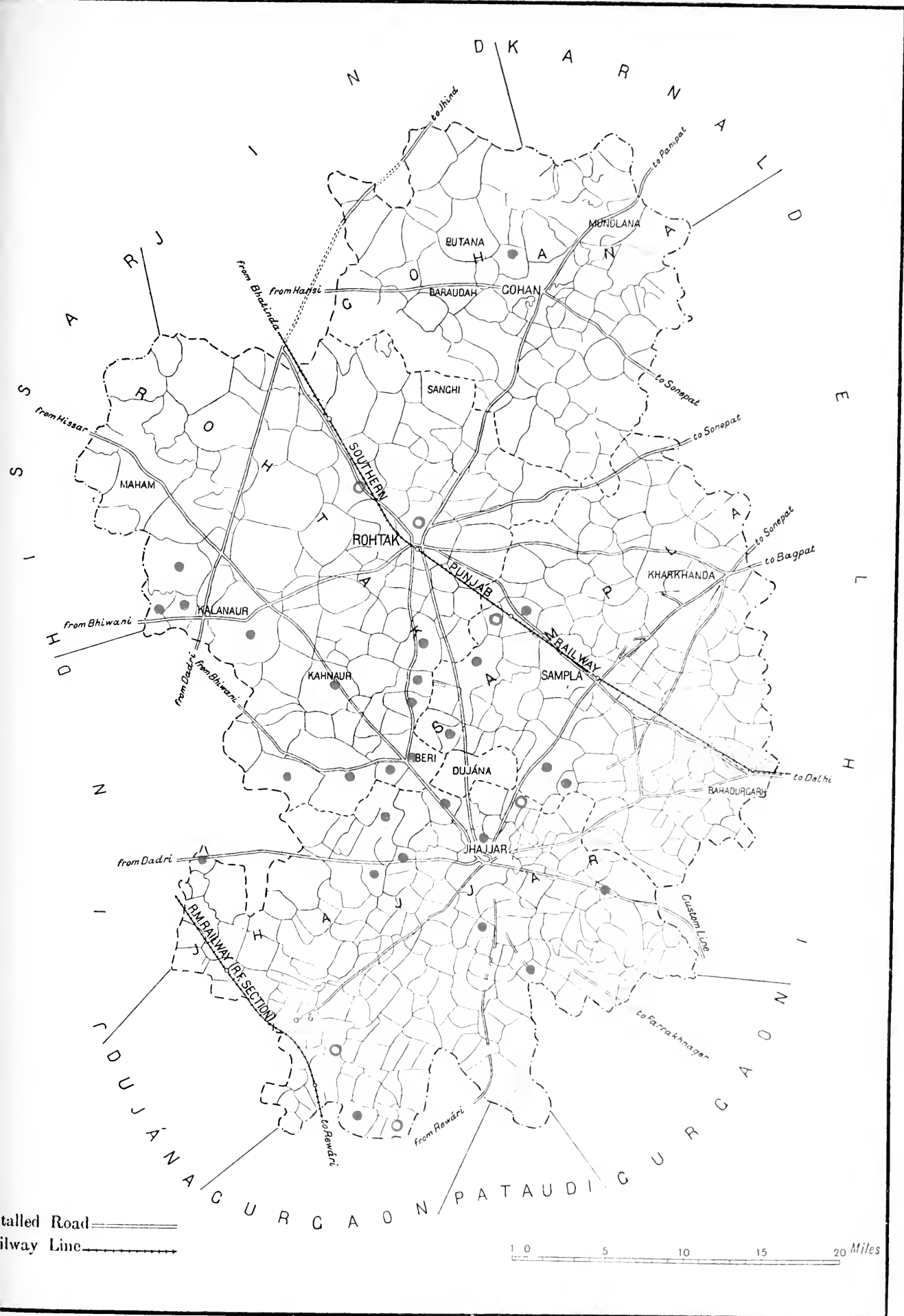
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March, 1904



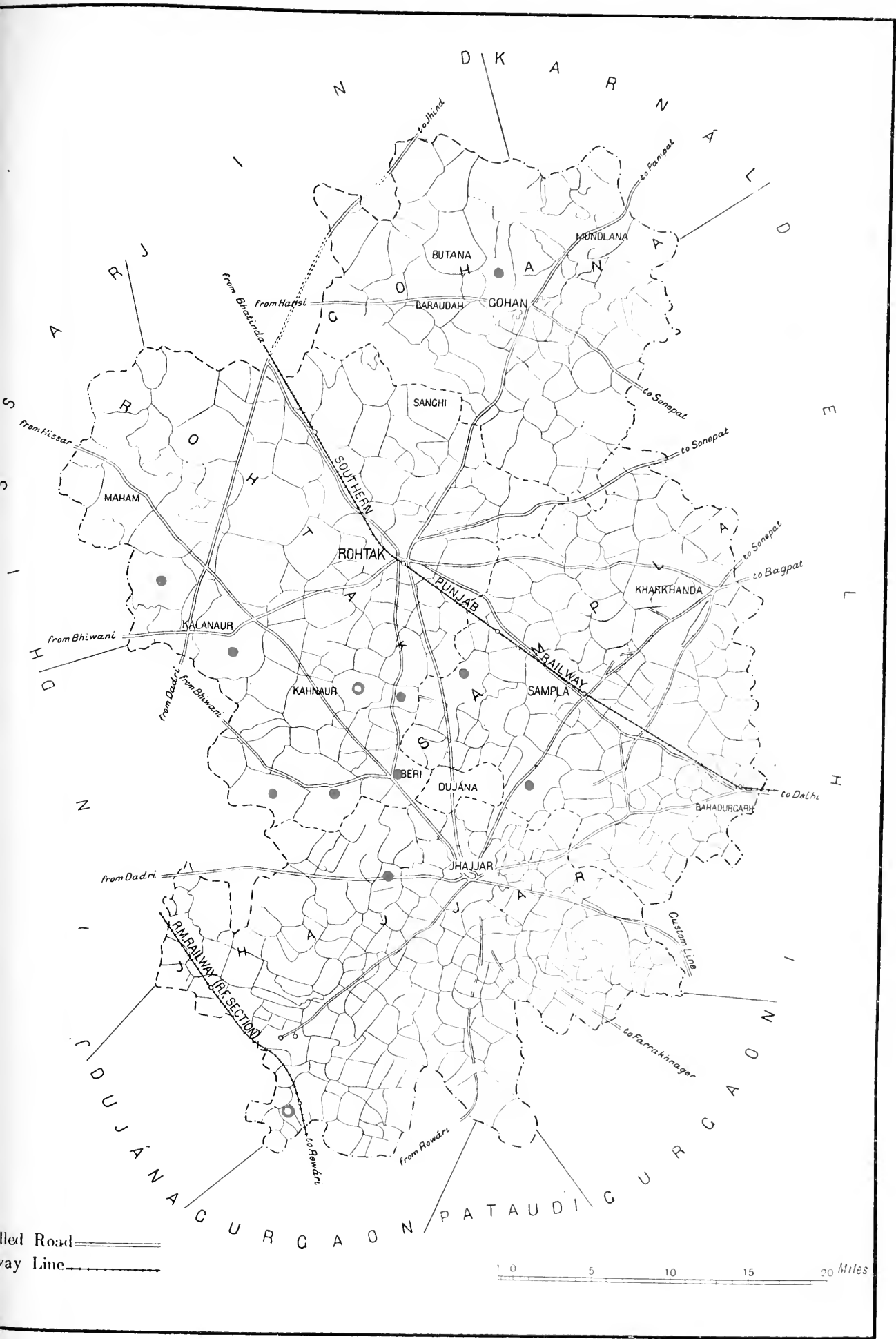
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April, 1904



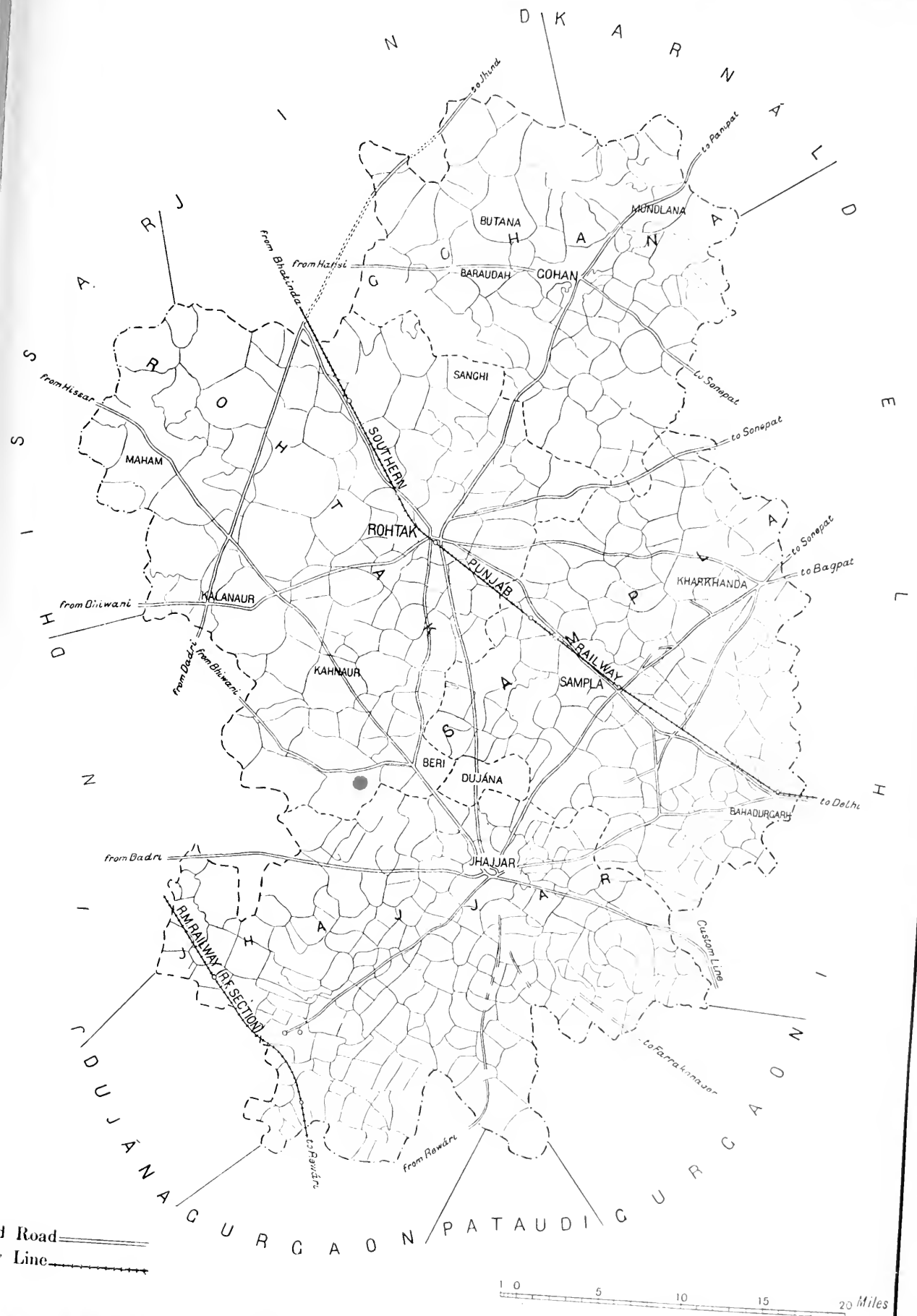
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May, 1904



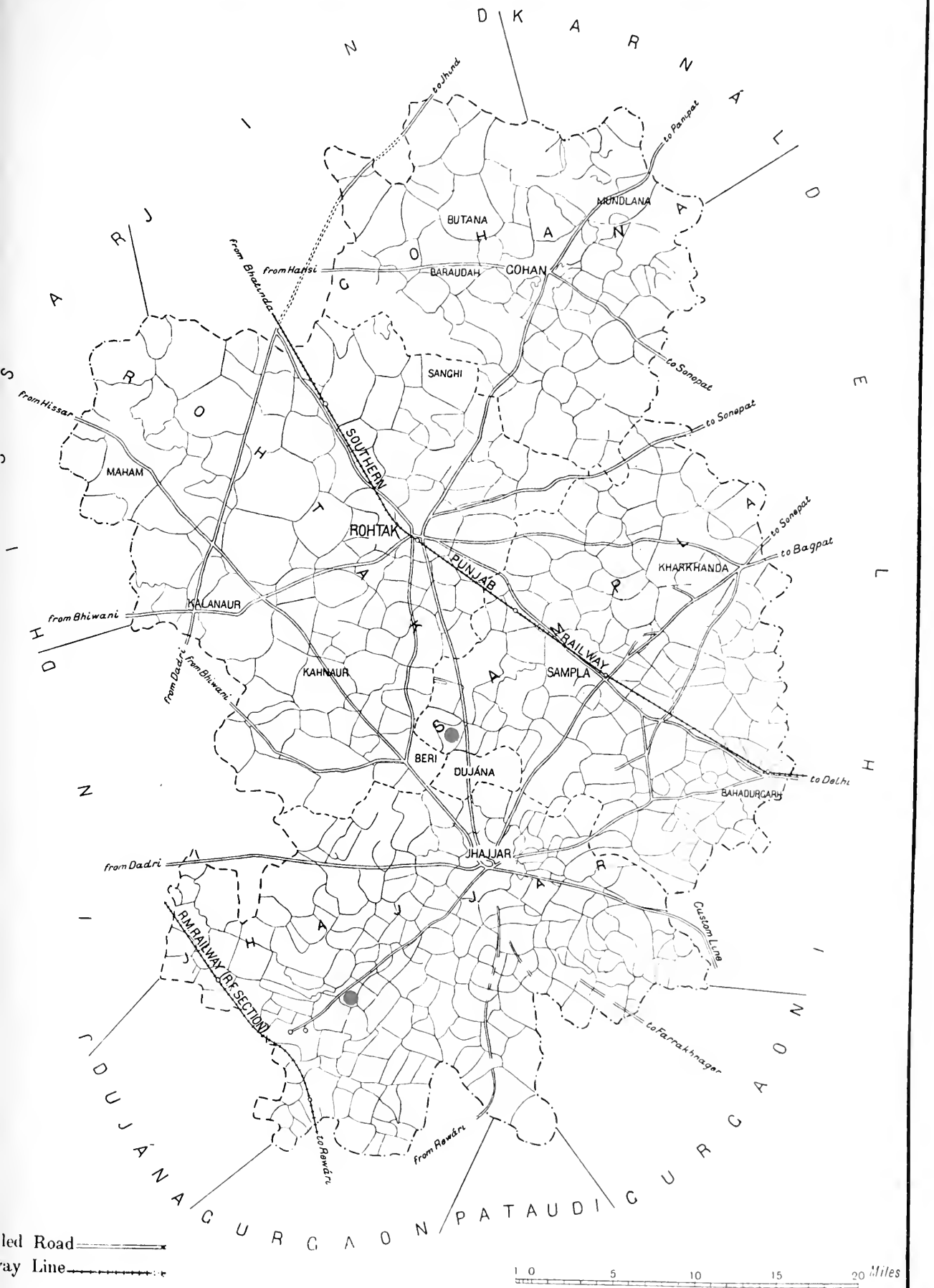
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June, 1904



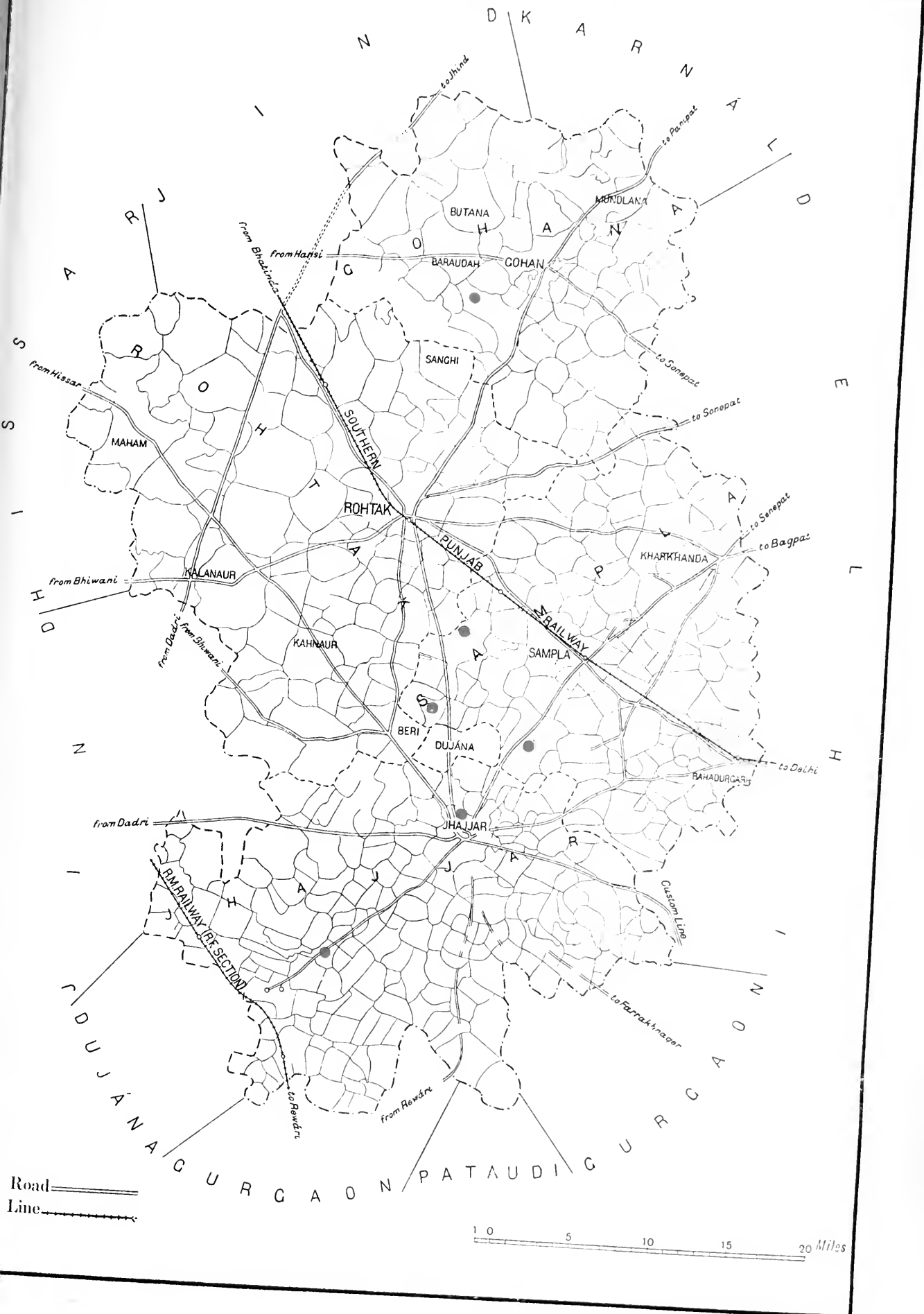
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July, 1904



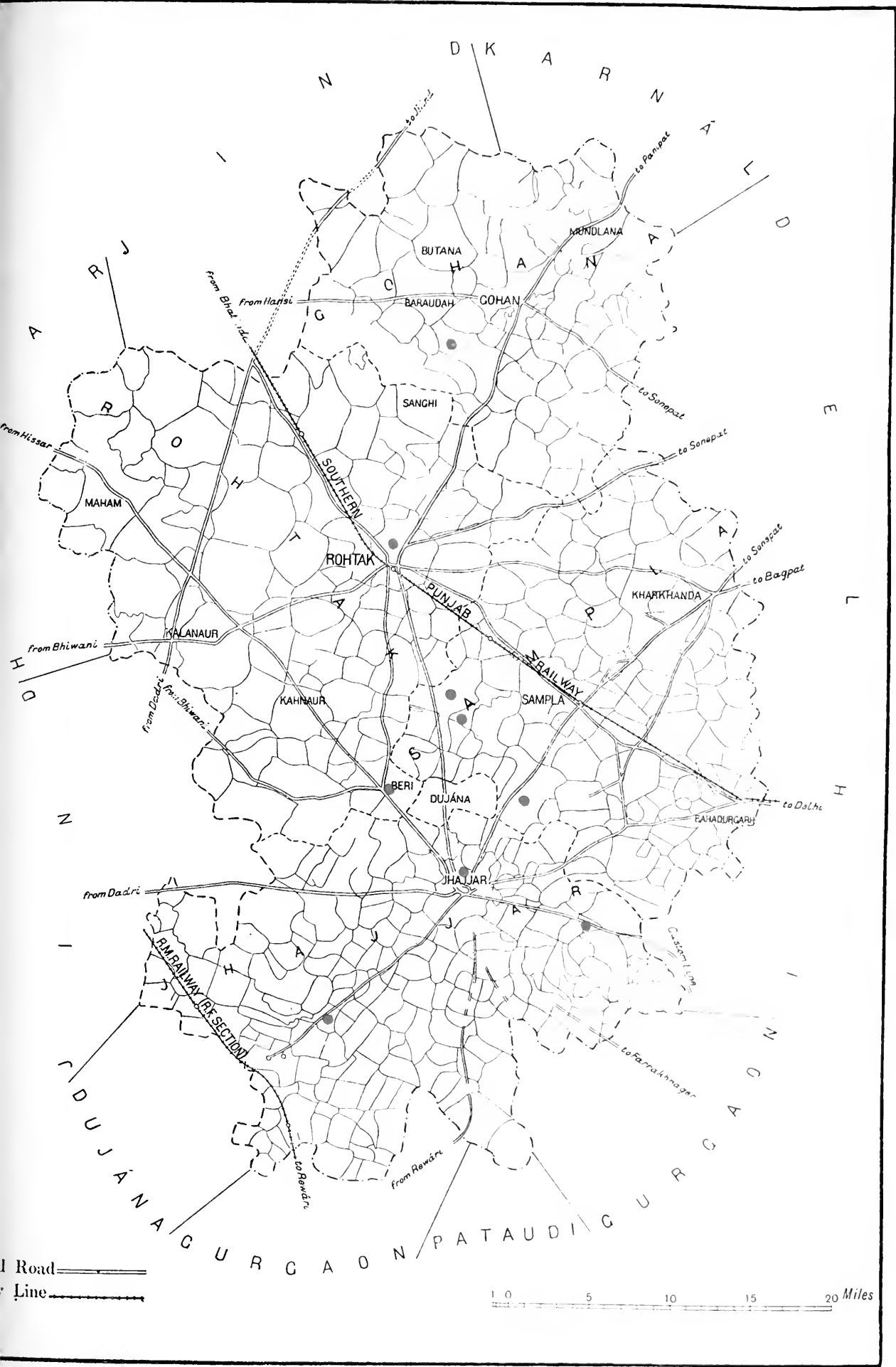
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August, 1904



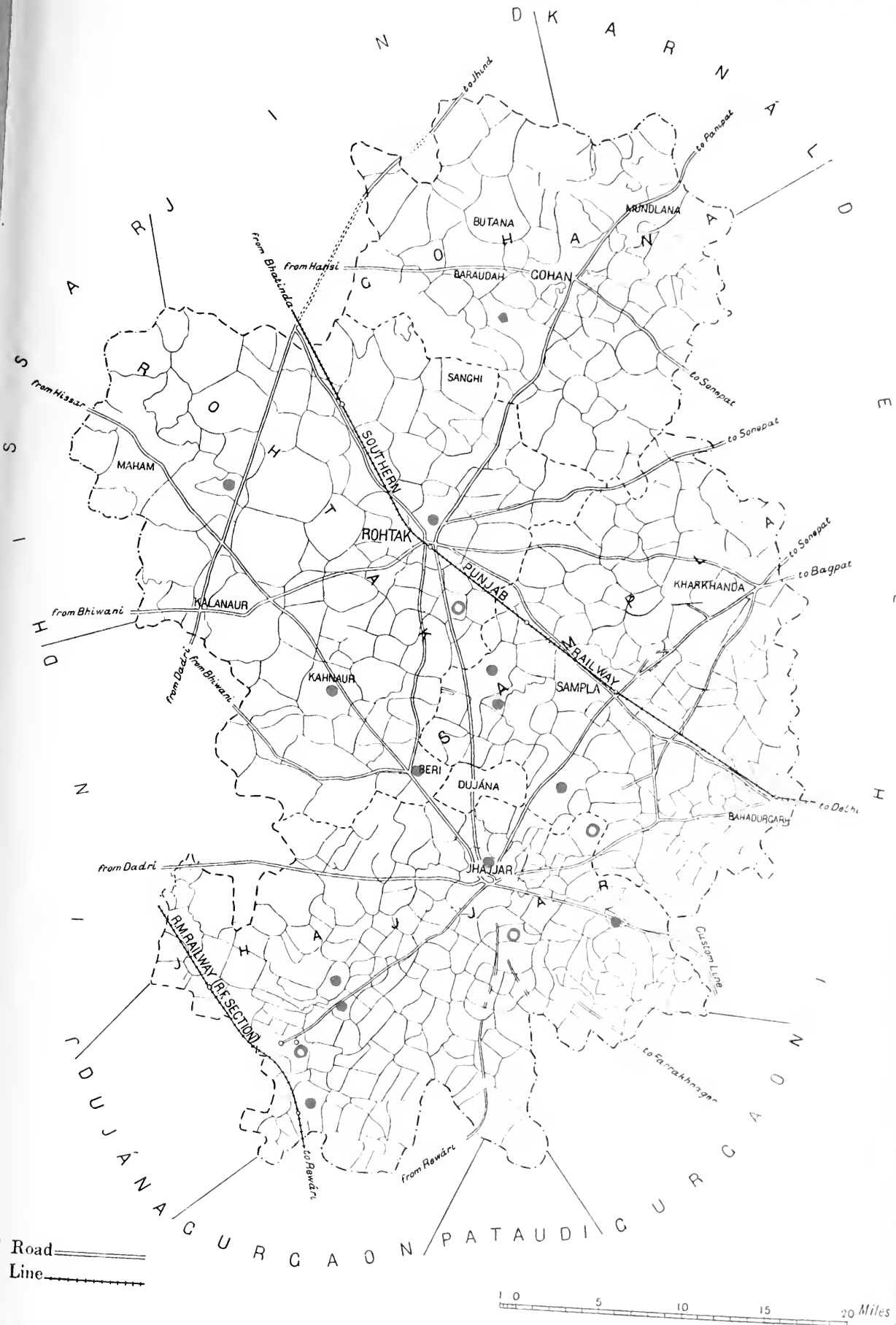
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September, 1904



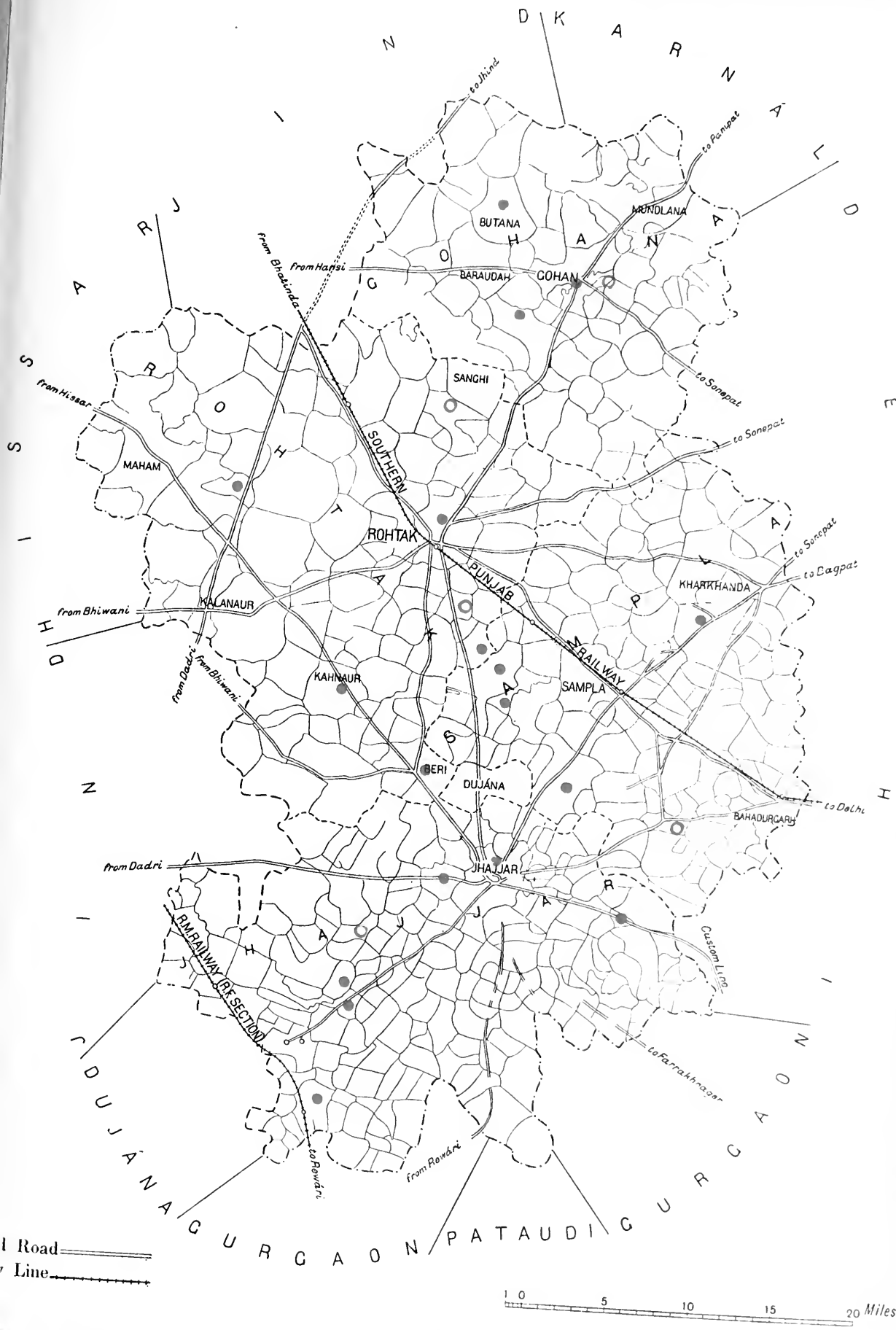
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October, 1904



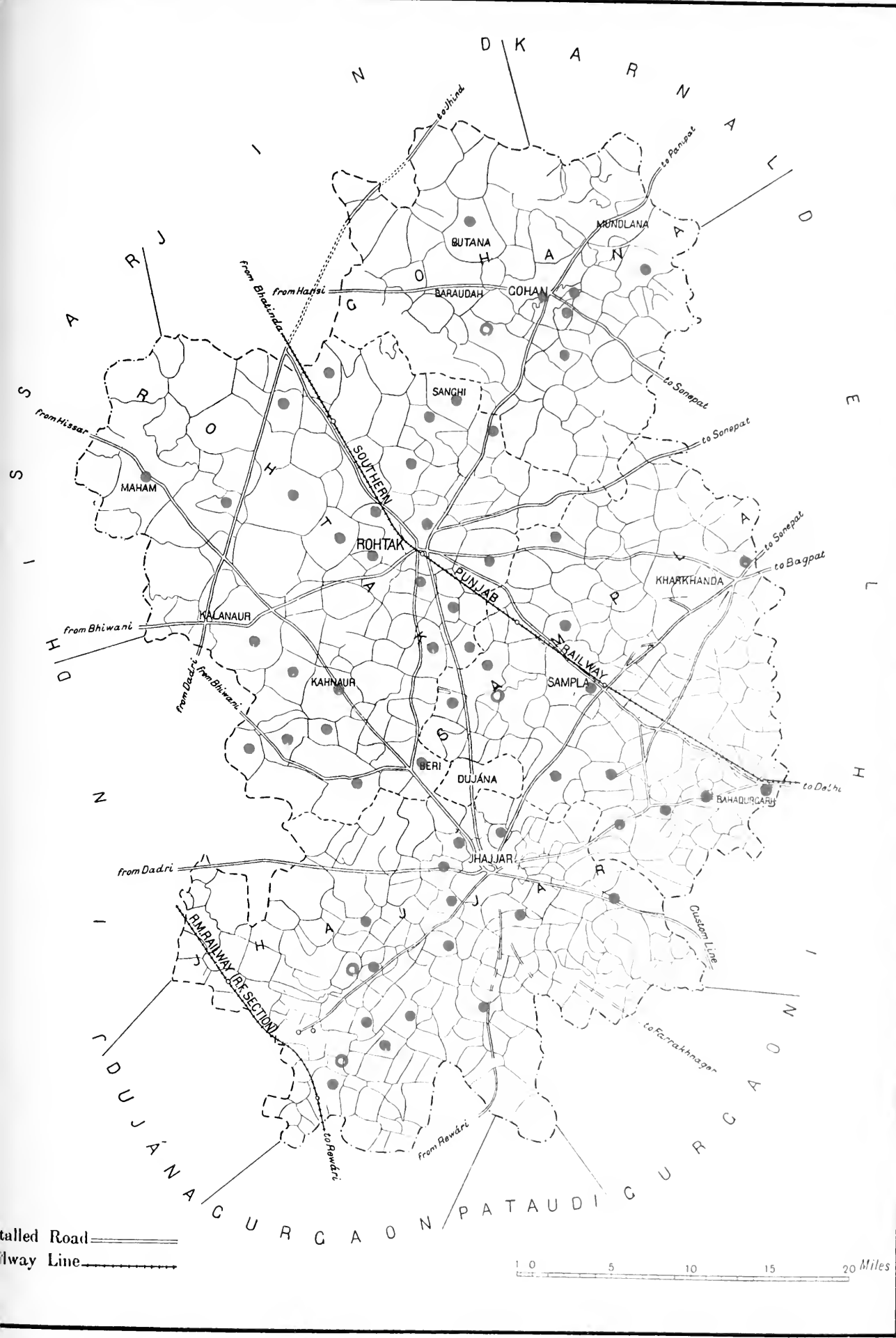
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November, 1904



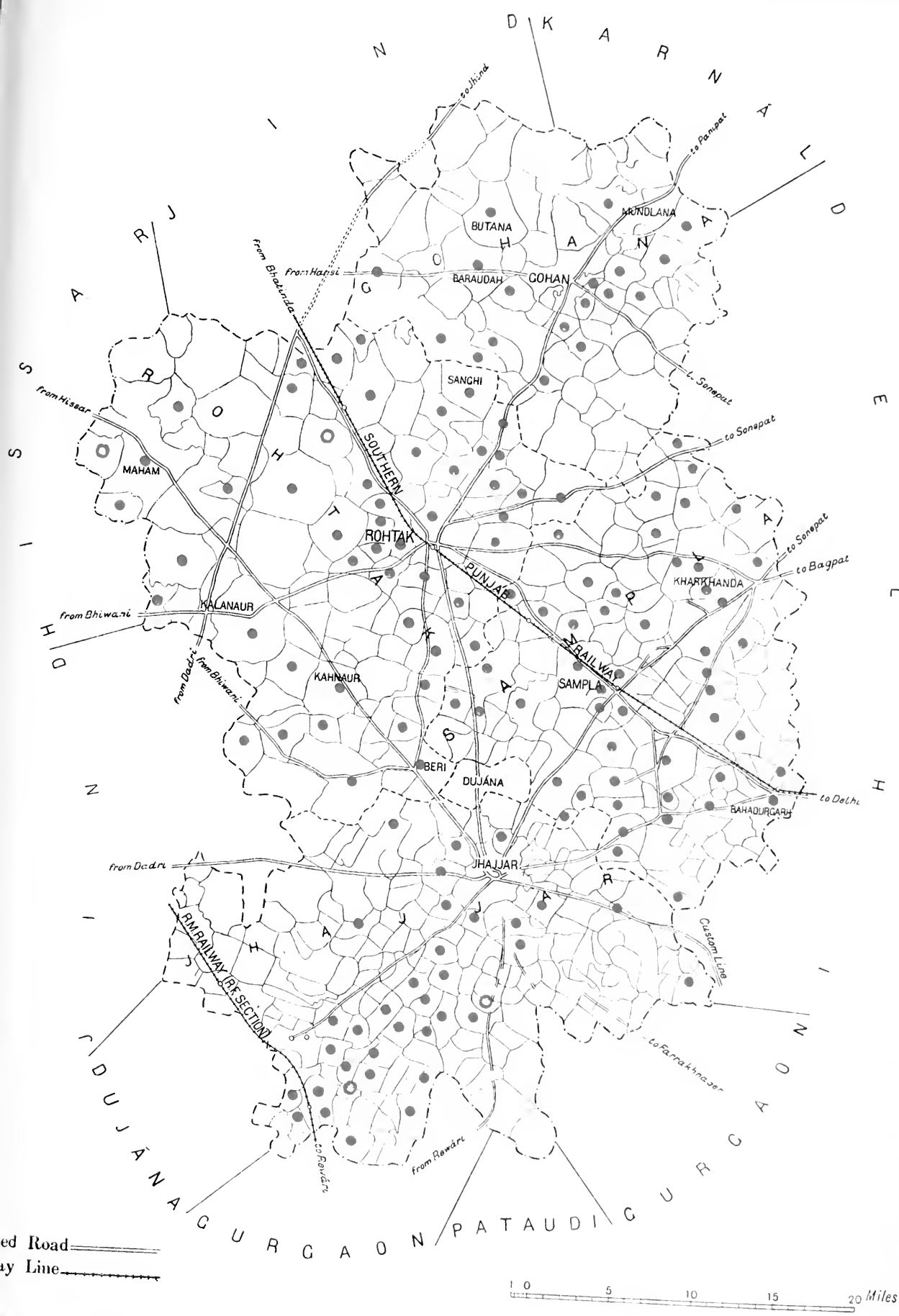
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December

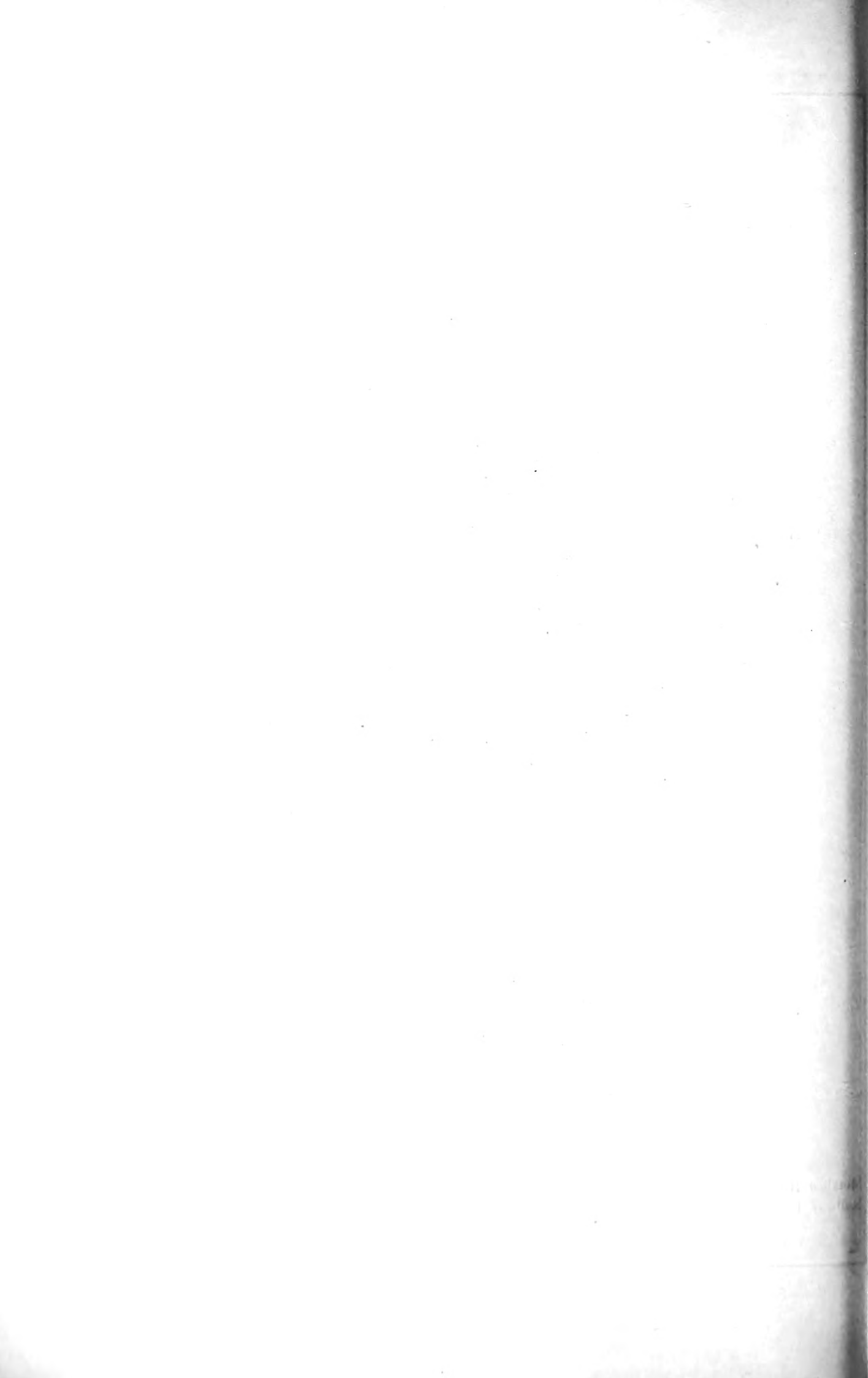


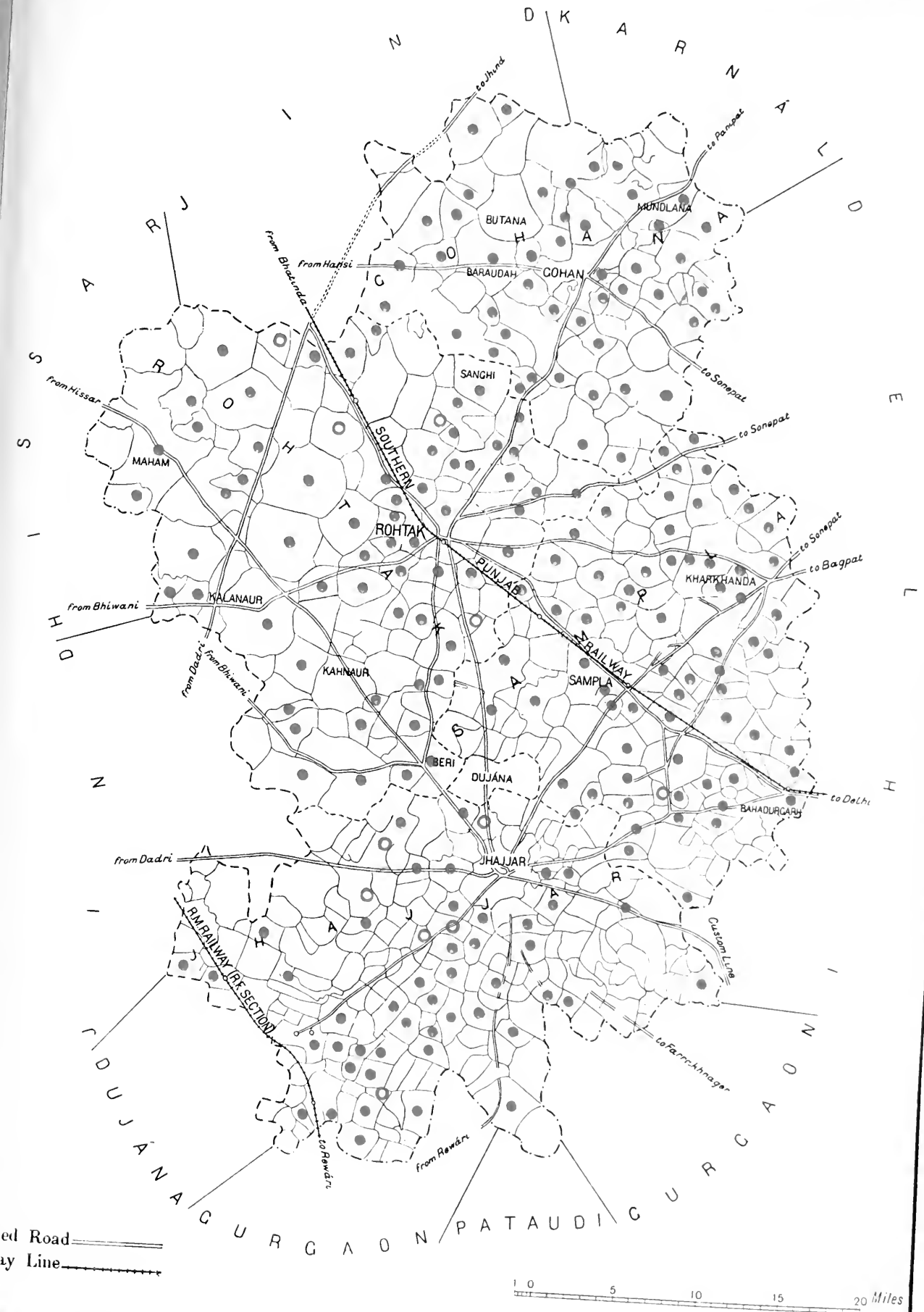
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January, 1905



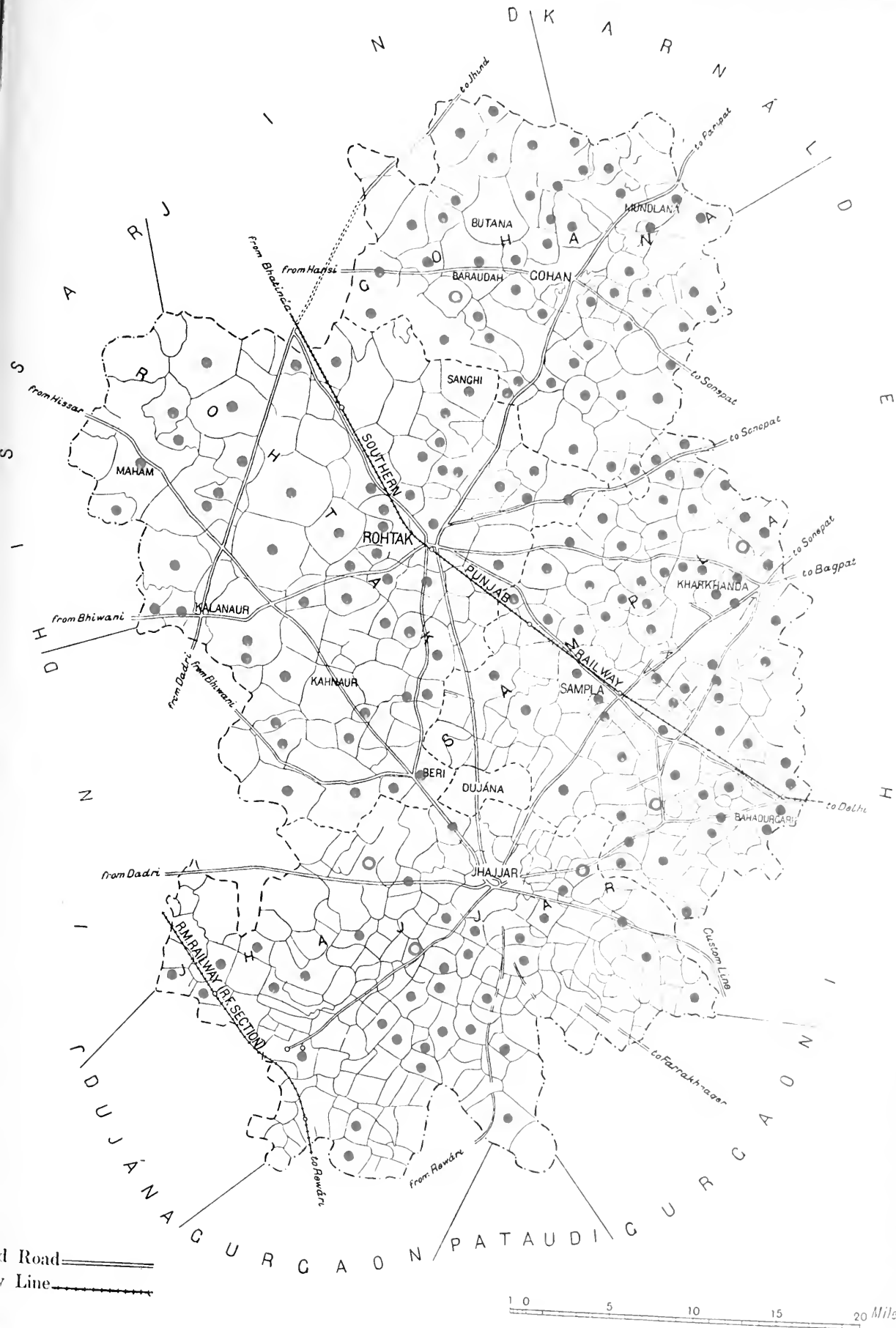
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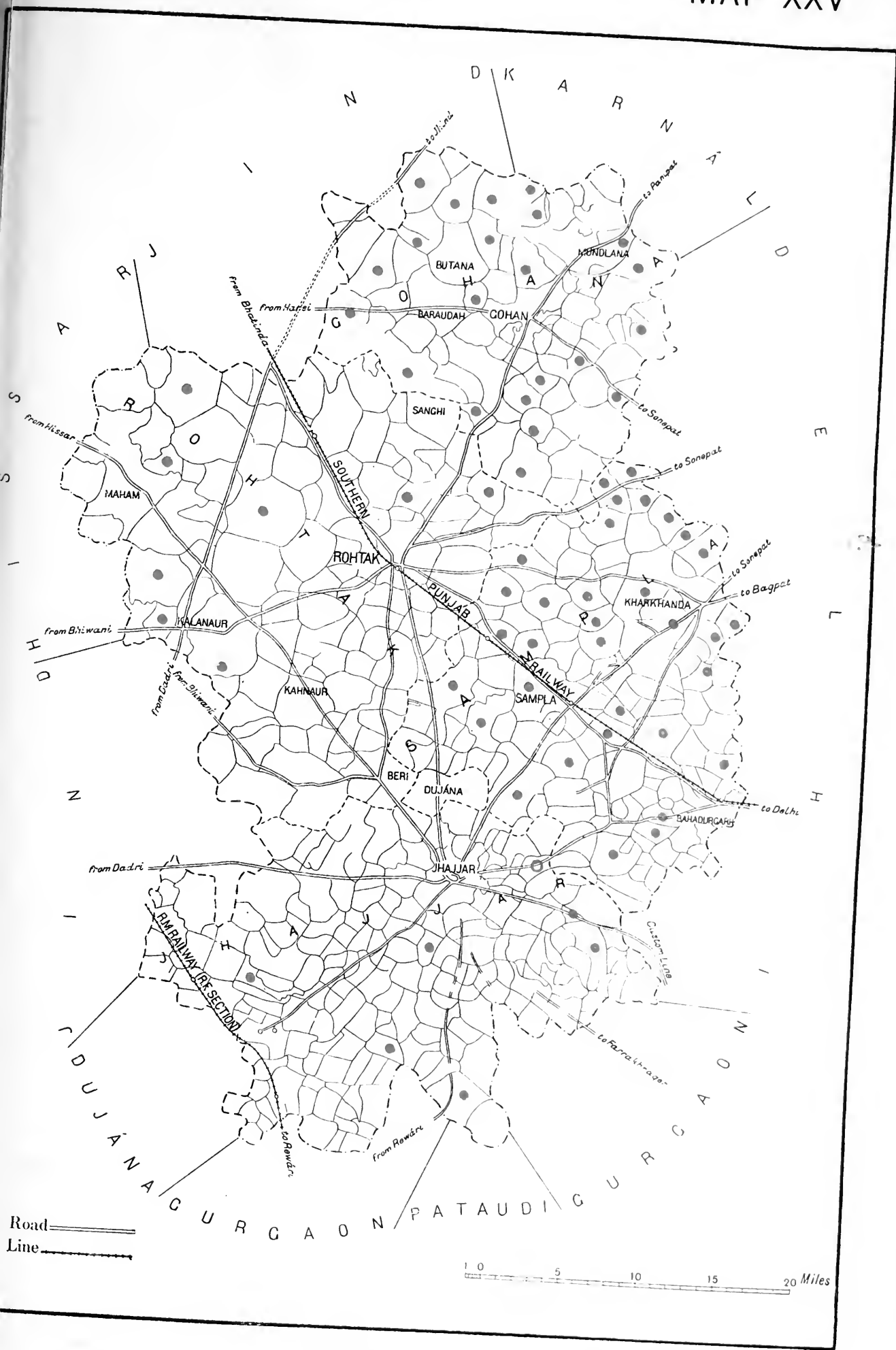


ROHTAK

April, 1905

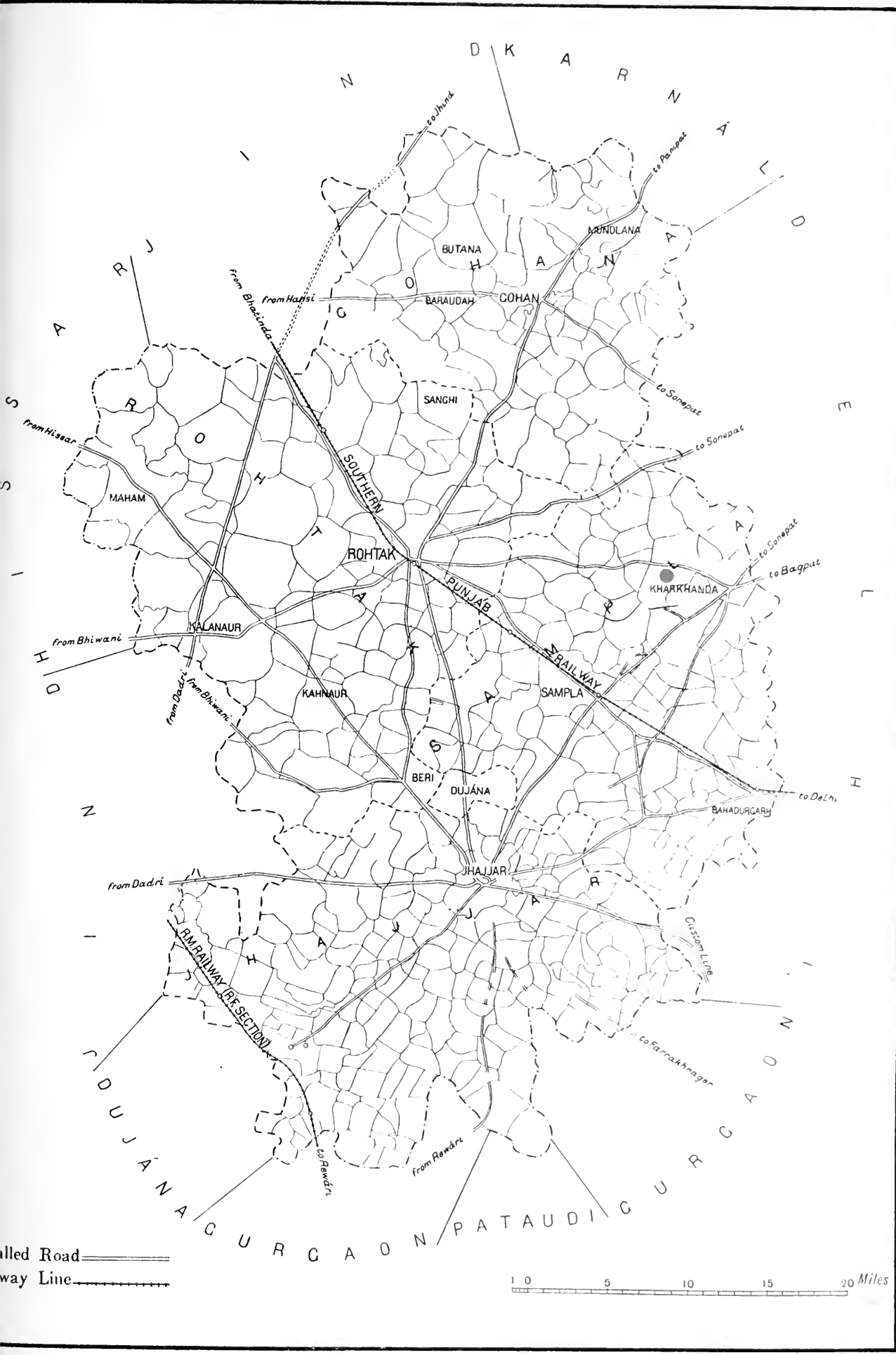


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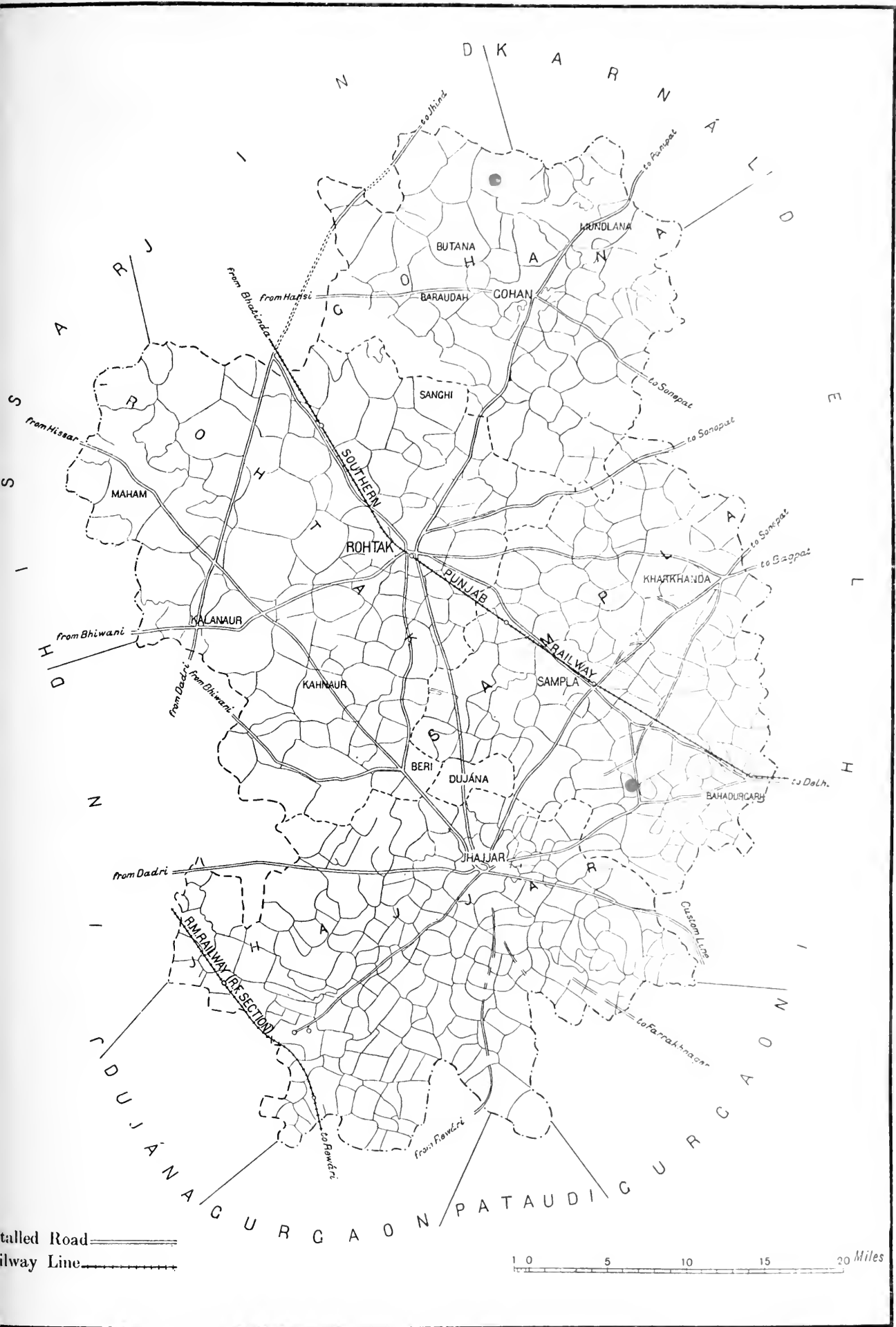
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June, 1905



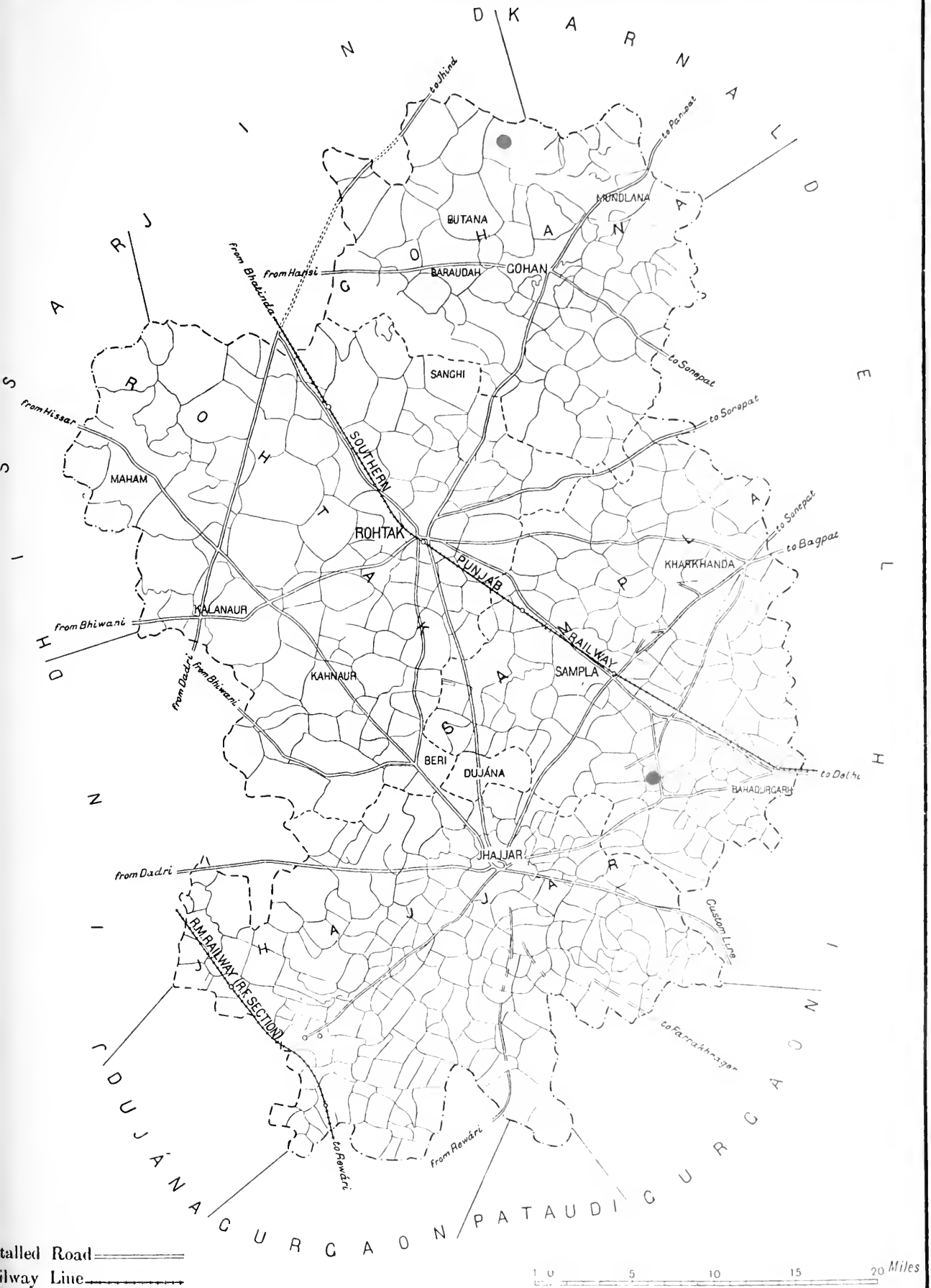
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July, 1905



ROHTAK

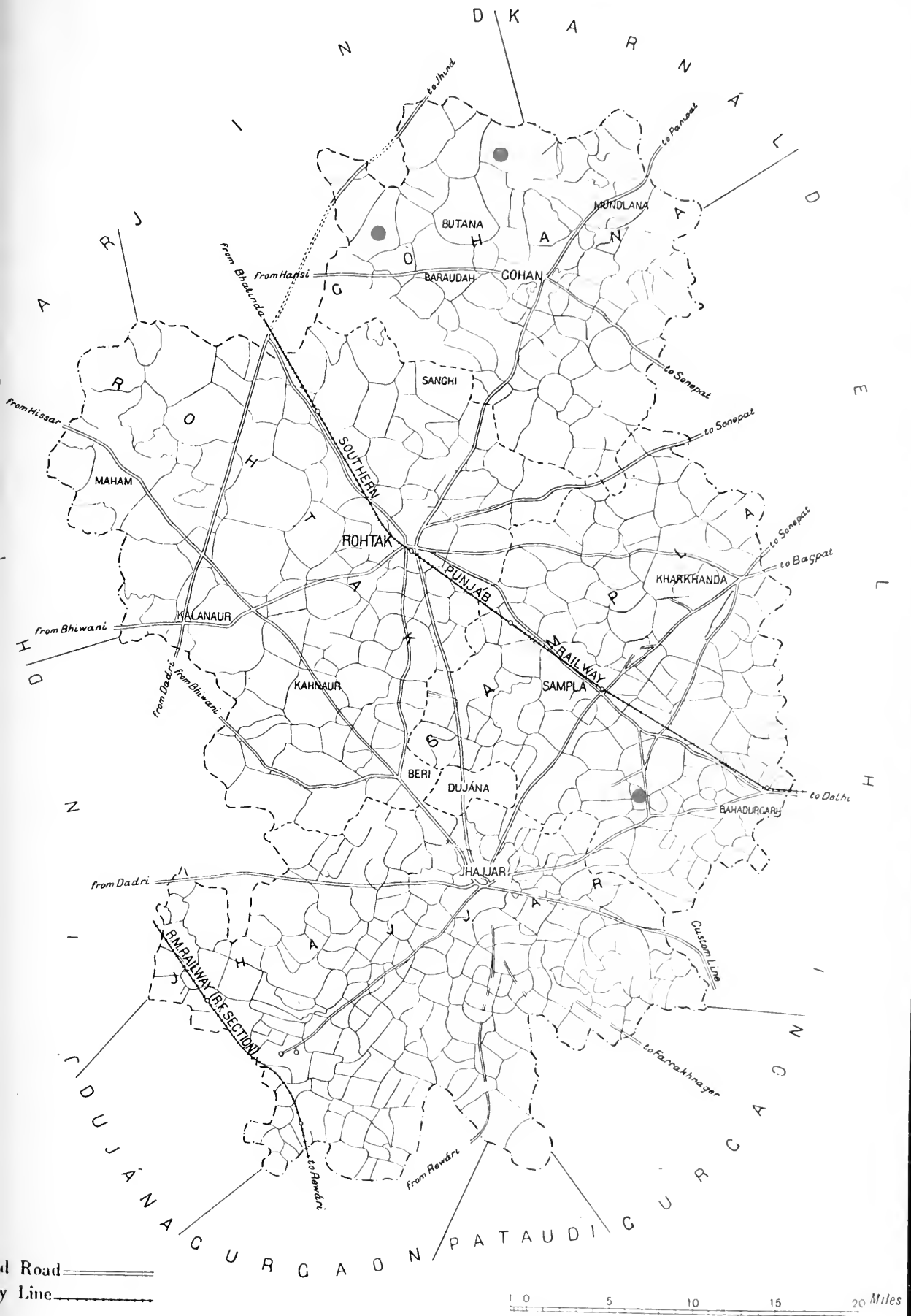
October, 1905



ROHTAK

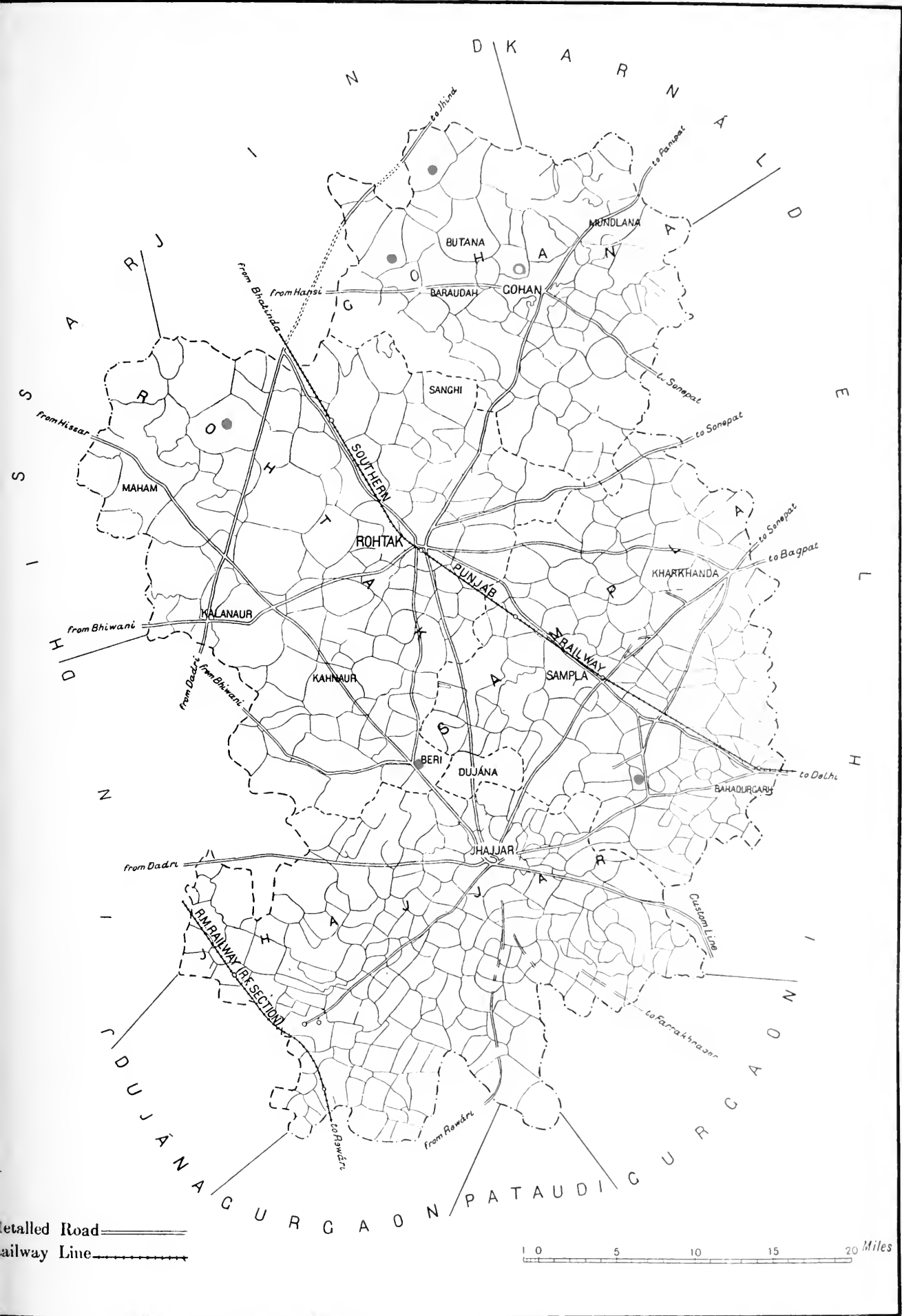
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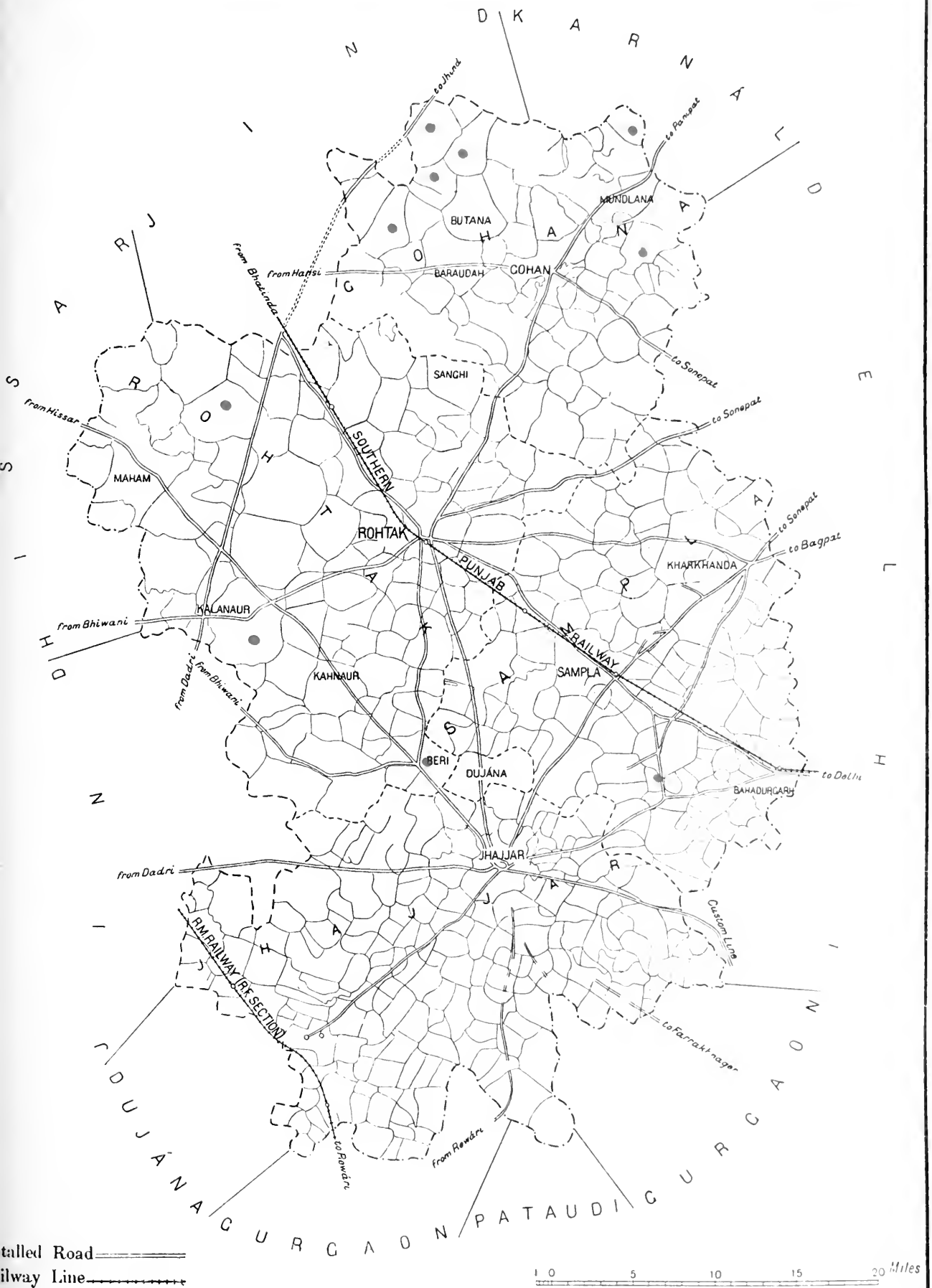
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December, 1905



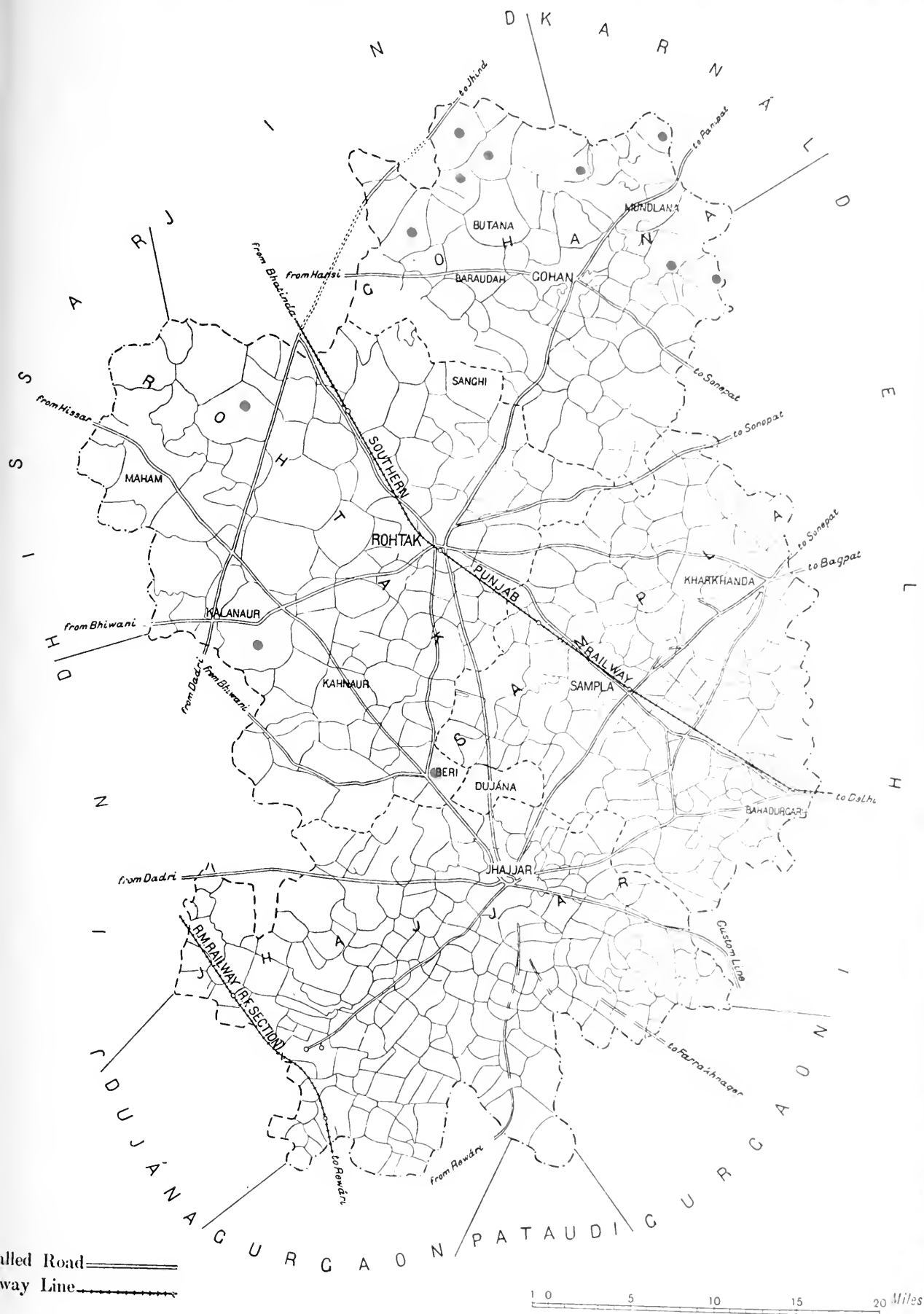
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January, 1906



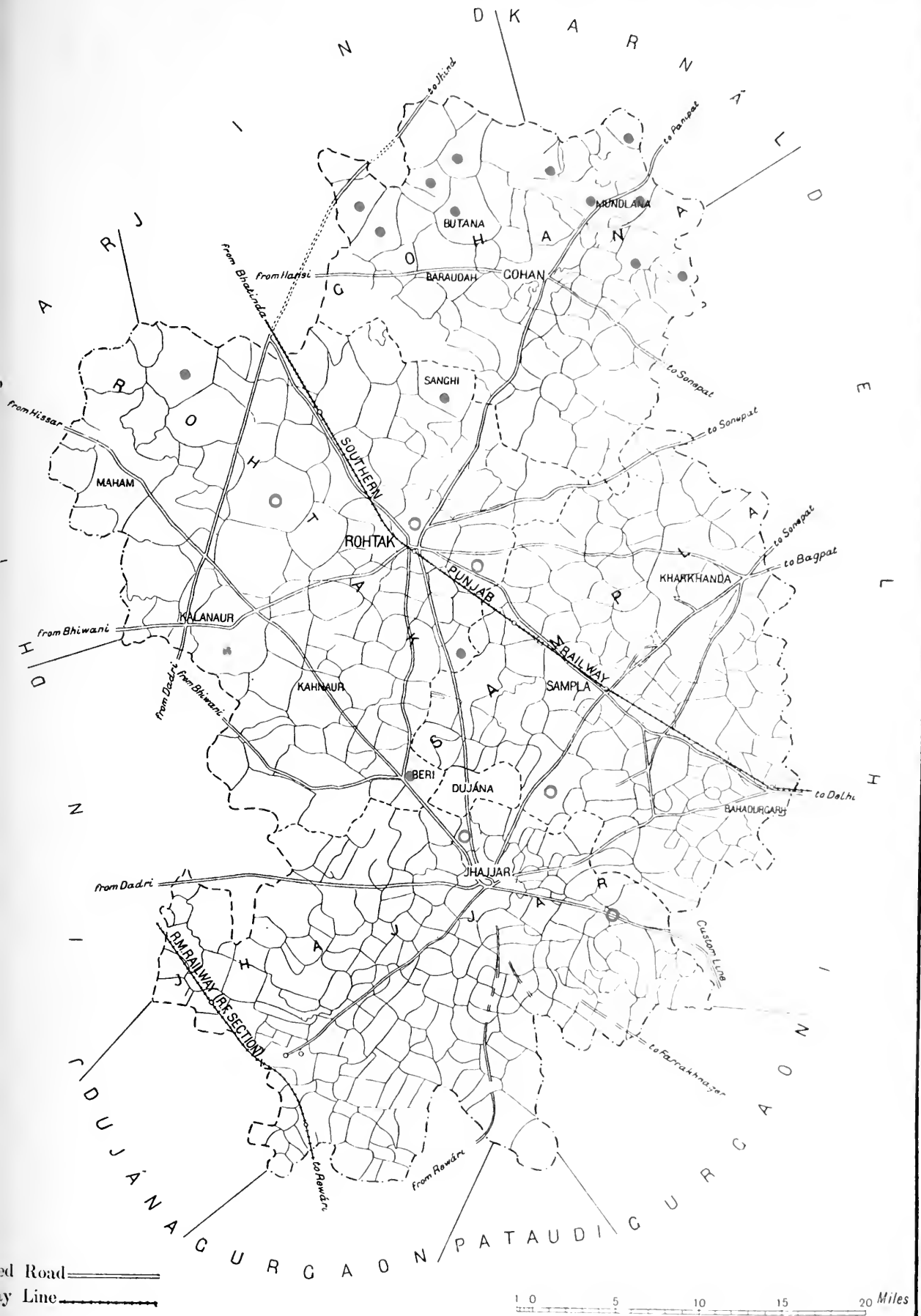
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February, 1906



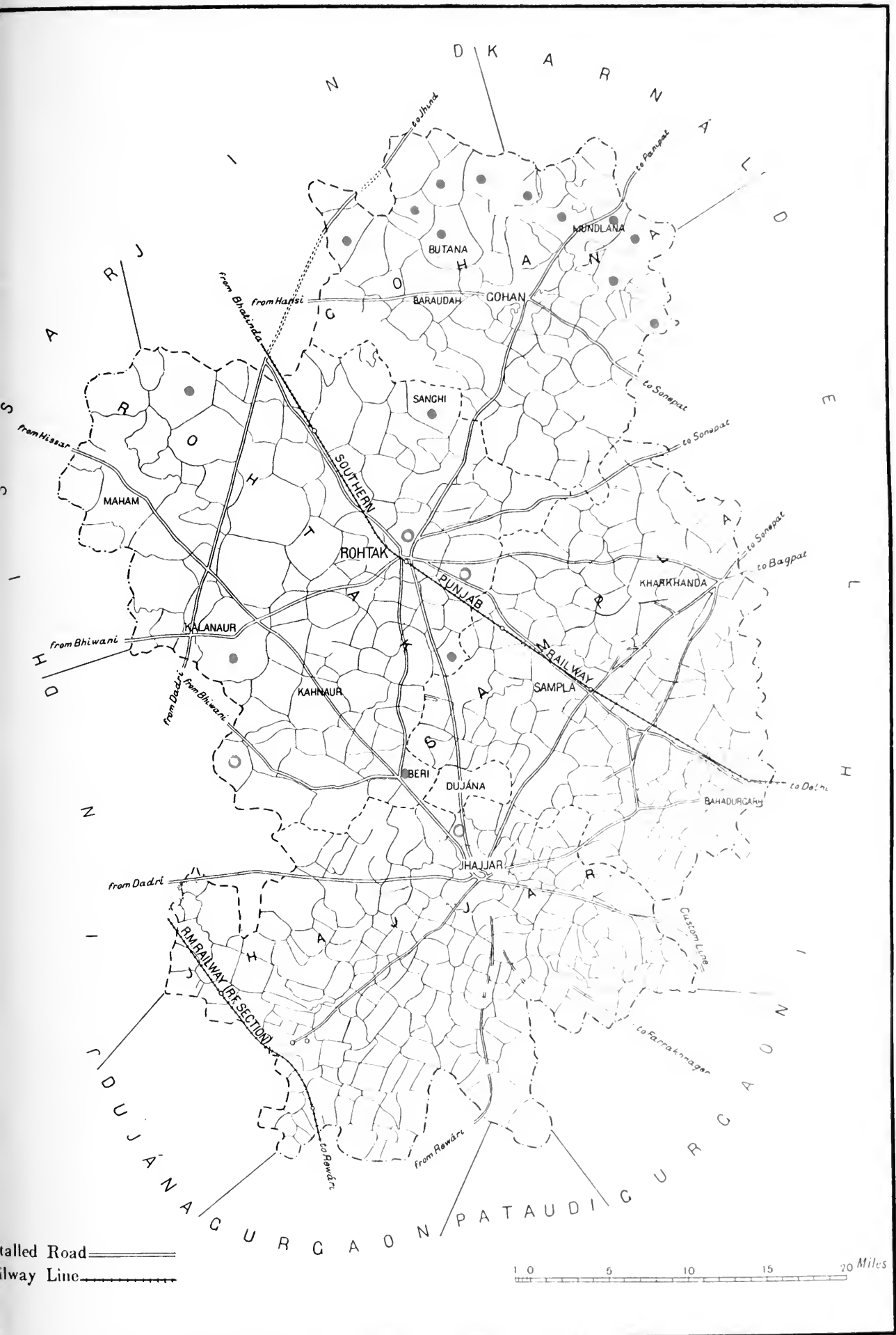
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March, 1906



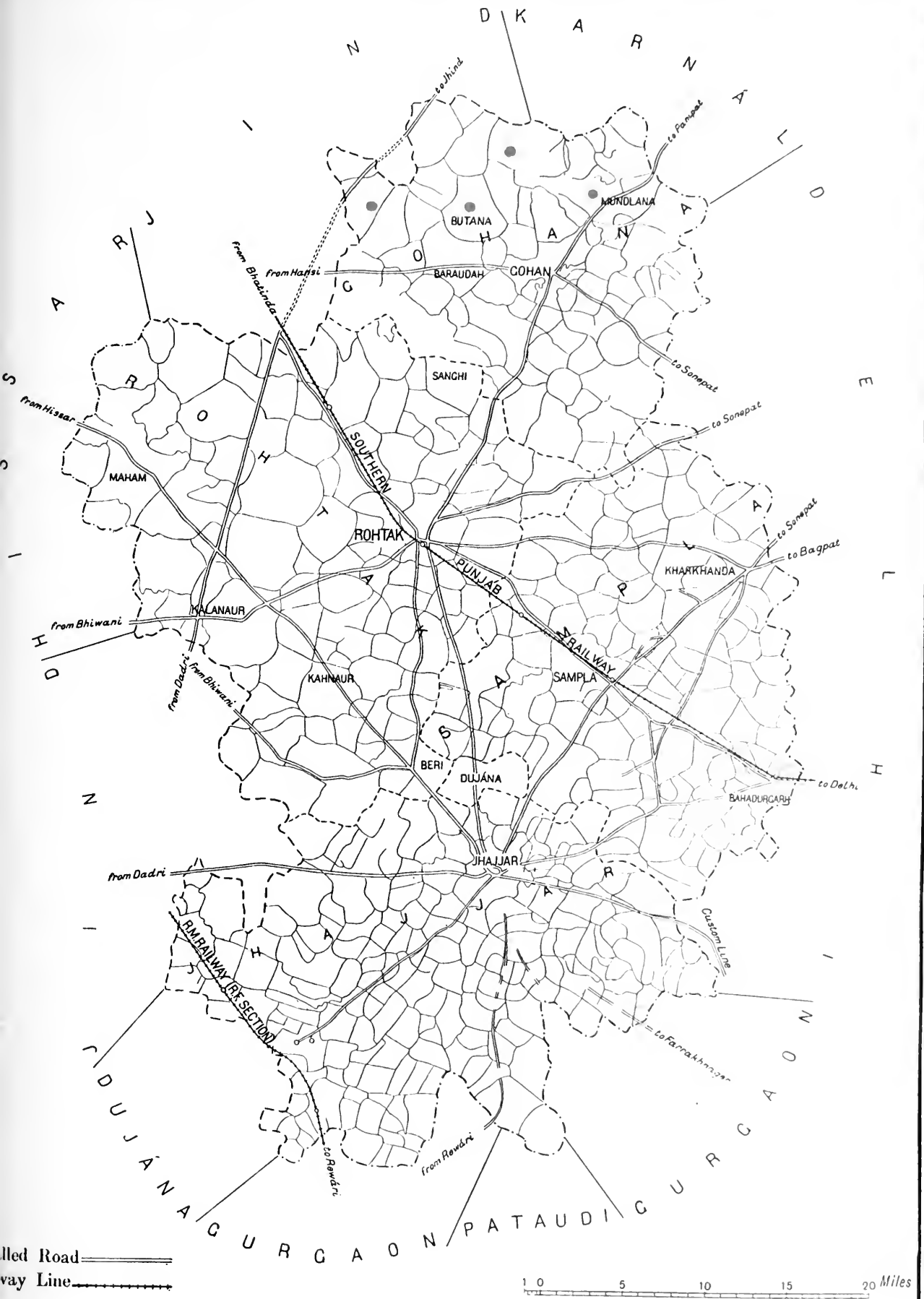
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April, 1906



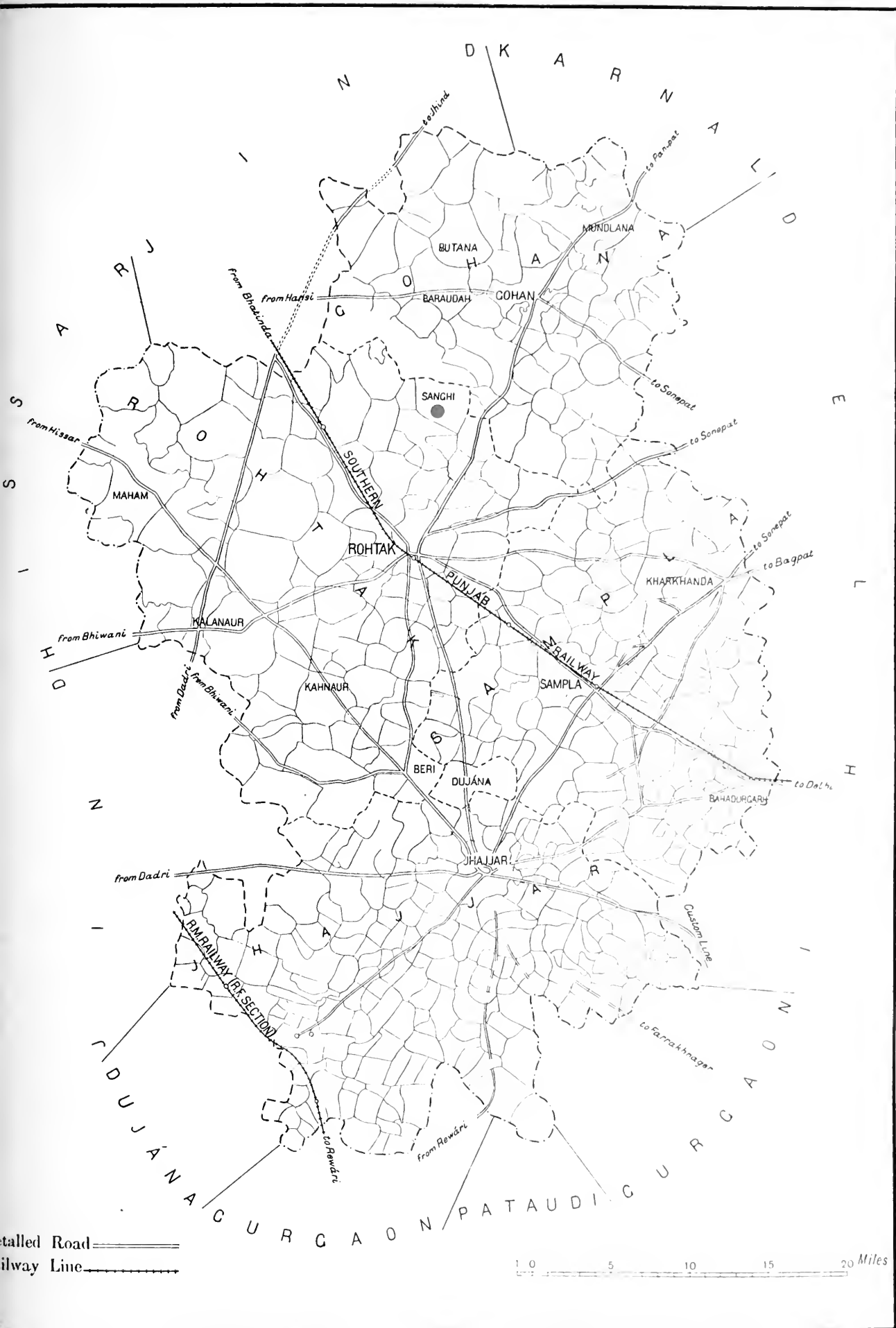
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May, 1906



ROHTAK

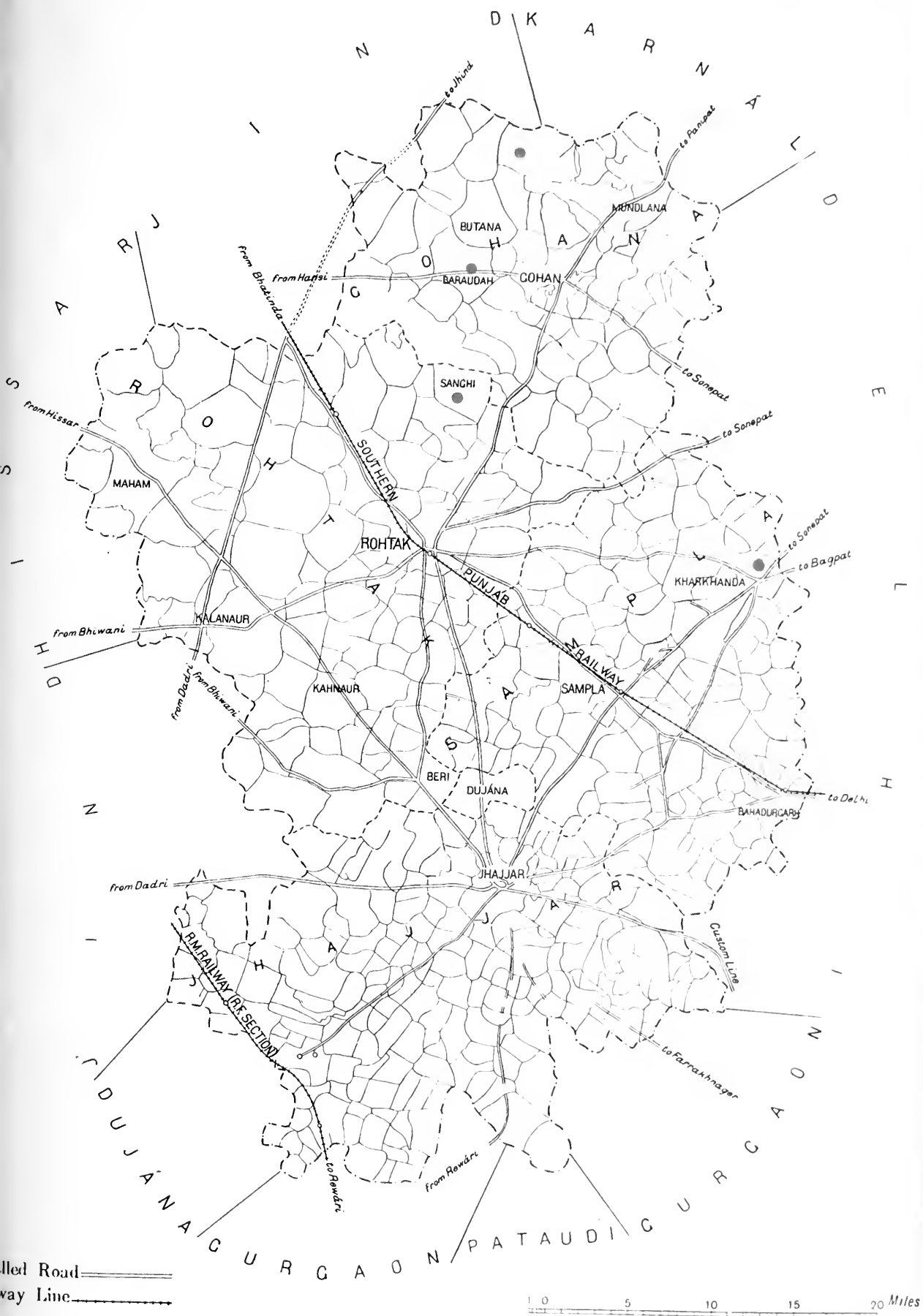
June, 1906



ROHTAK

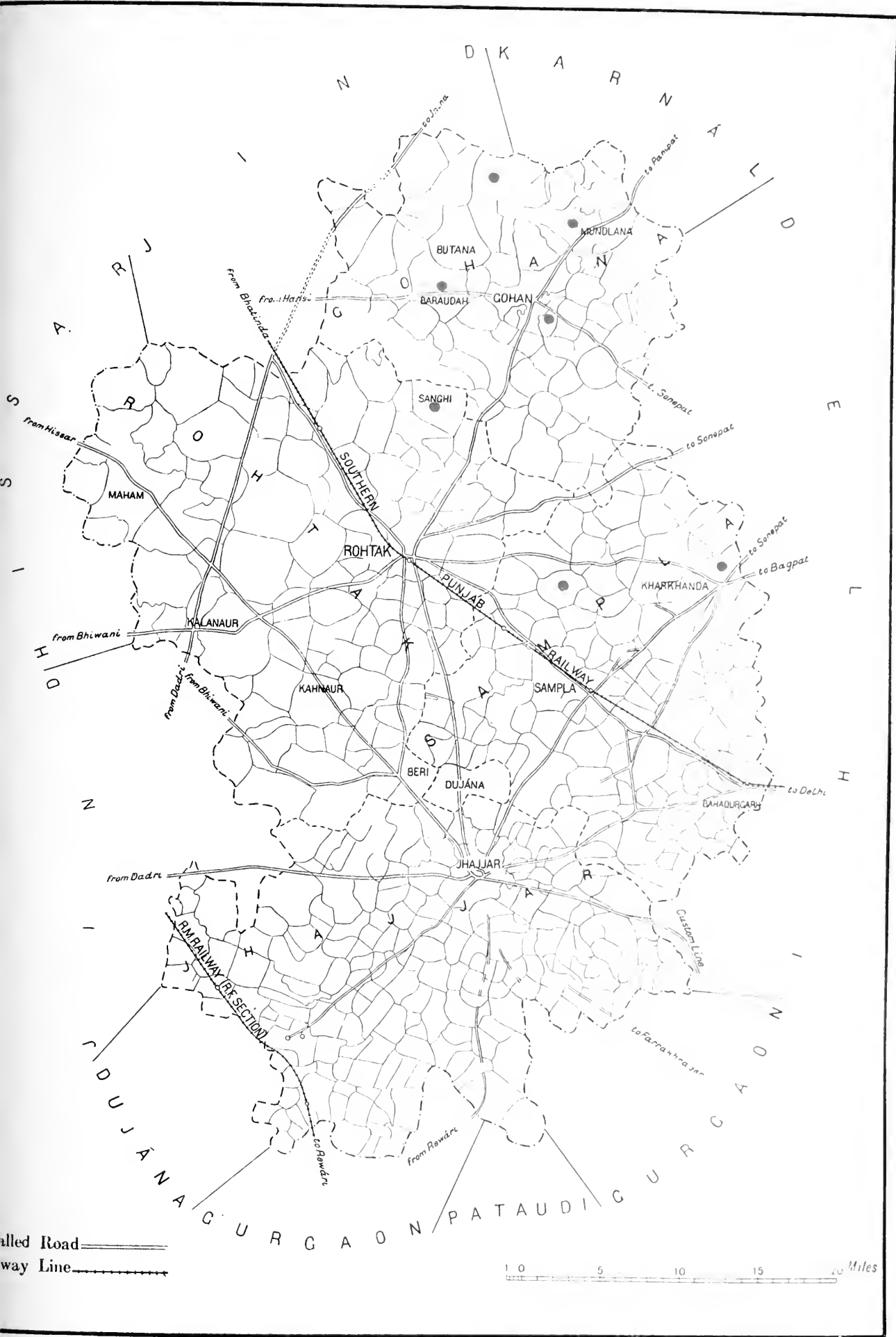
August, 1906





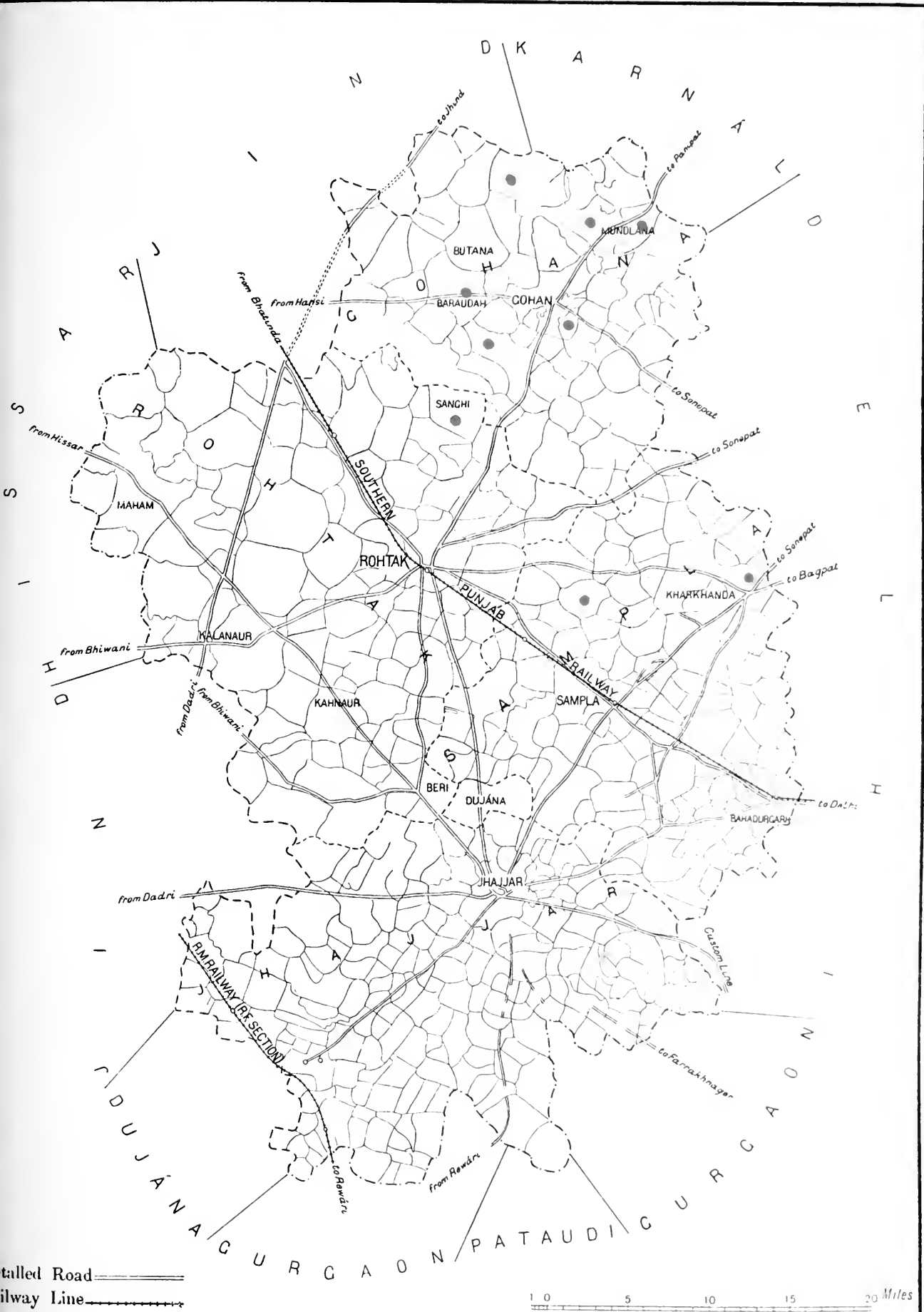
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September, 1906

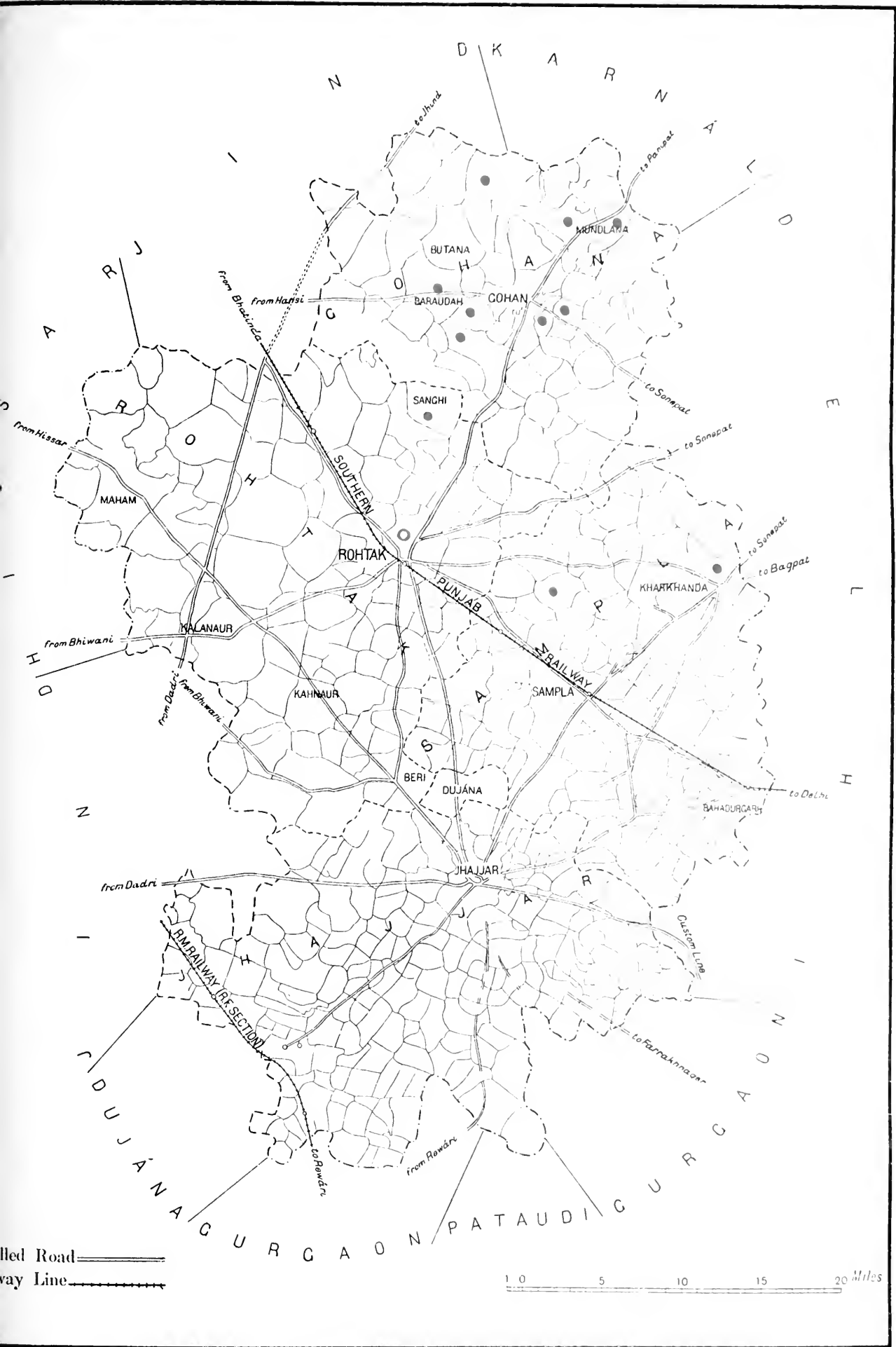


ROHTAK

October, 1906

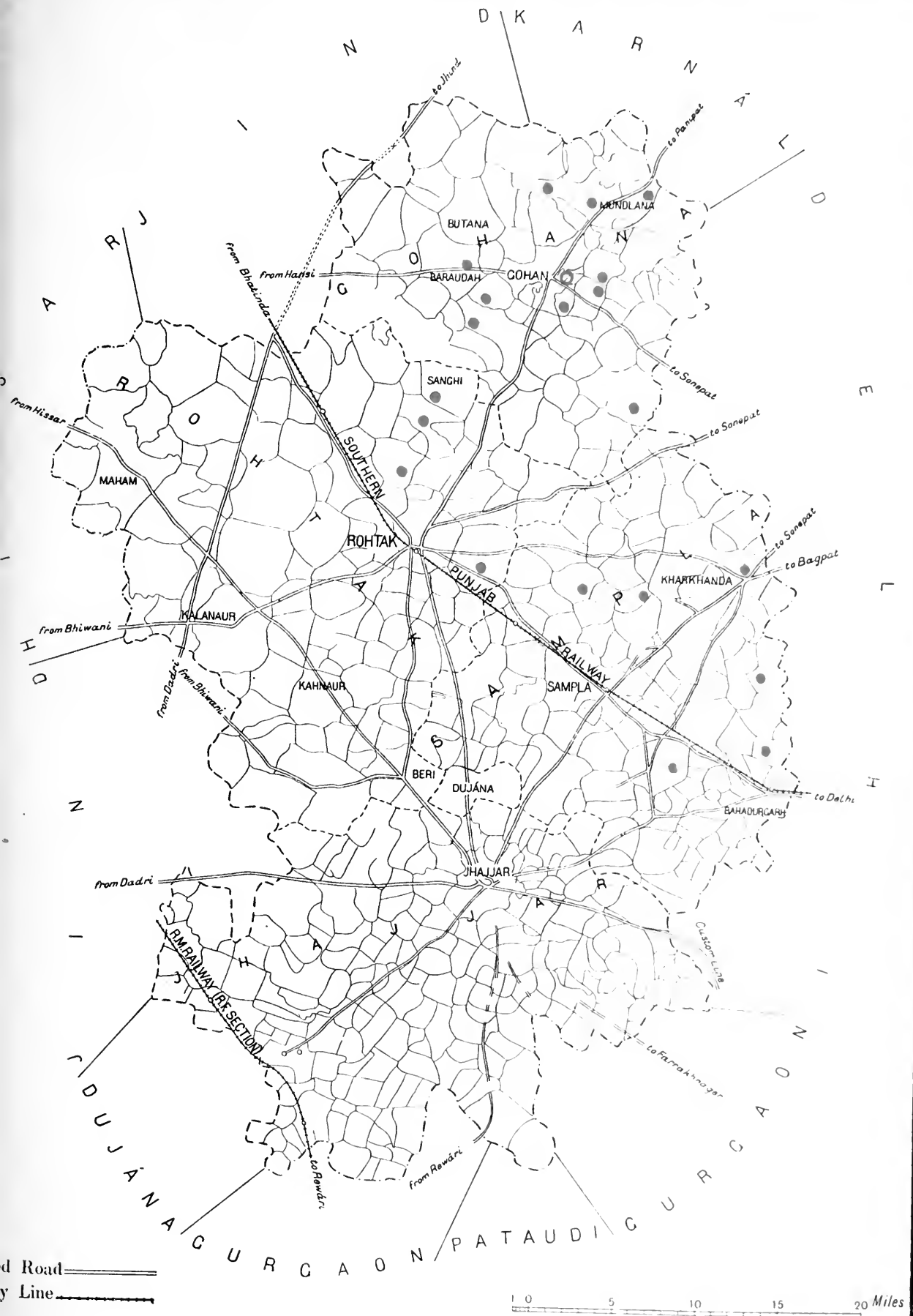


ROHTAK



ROHTAK

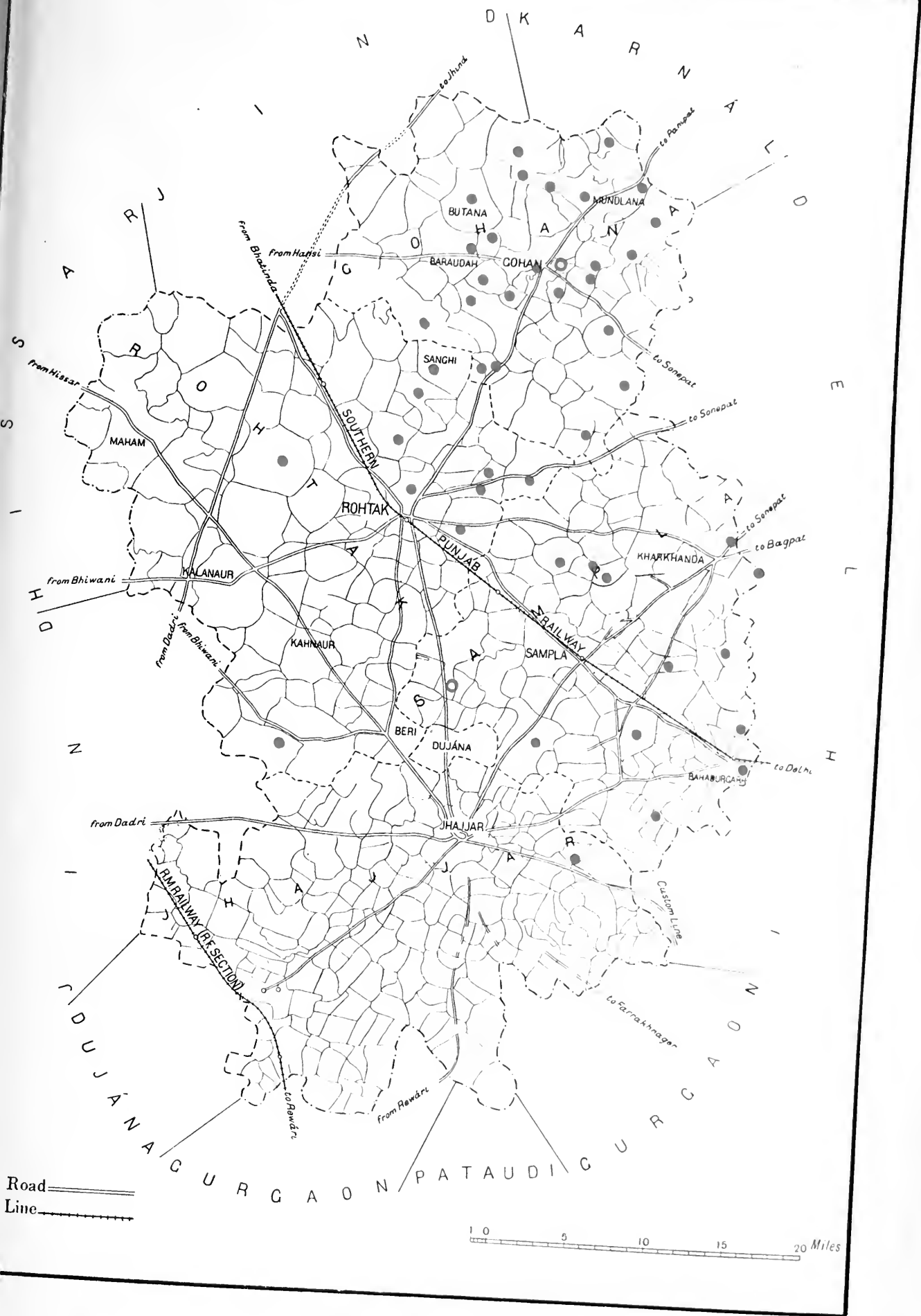
December, 1906



ROHTAK

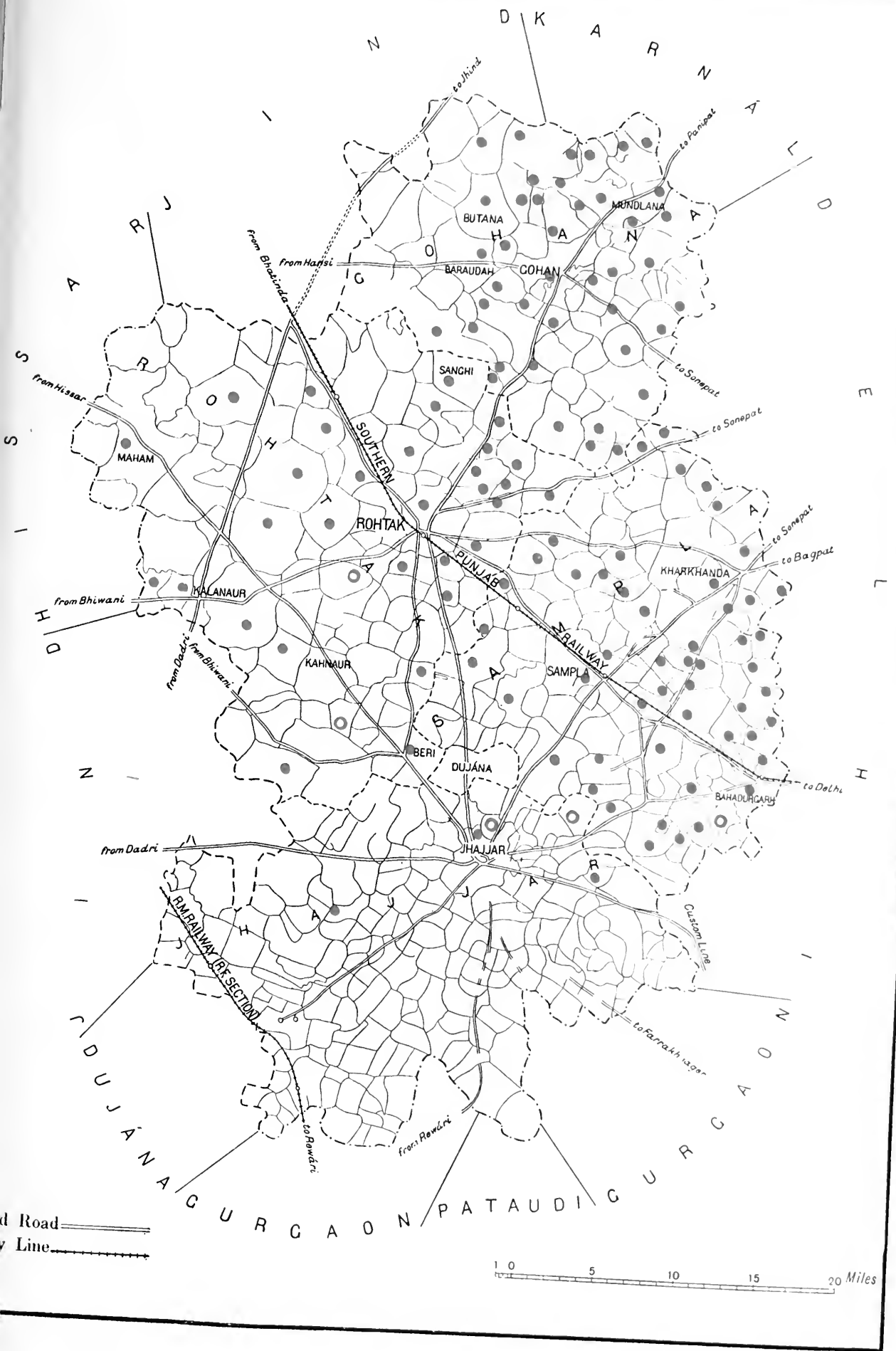
January, 1907

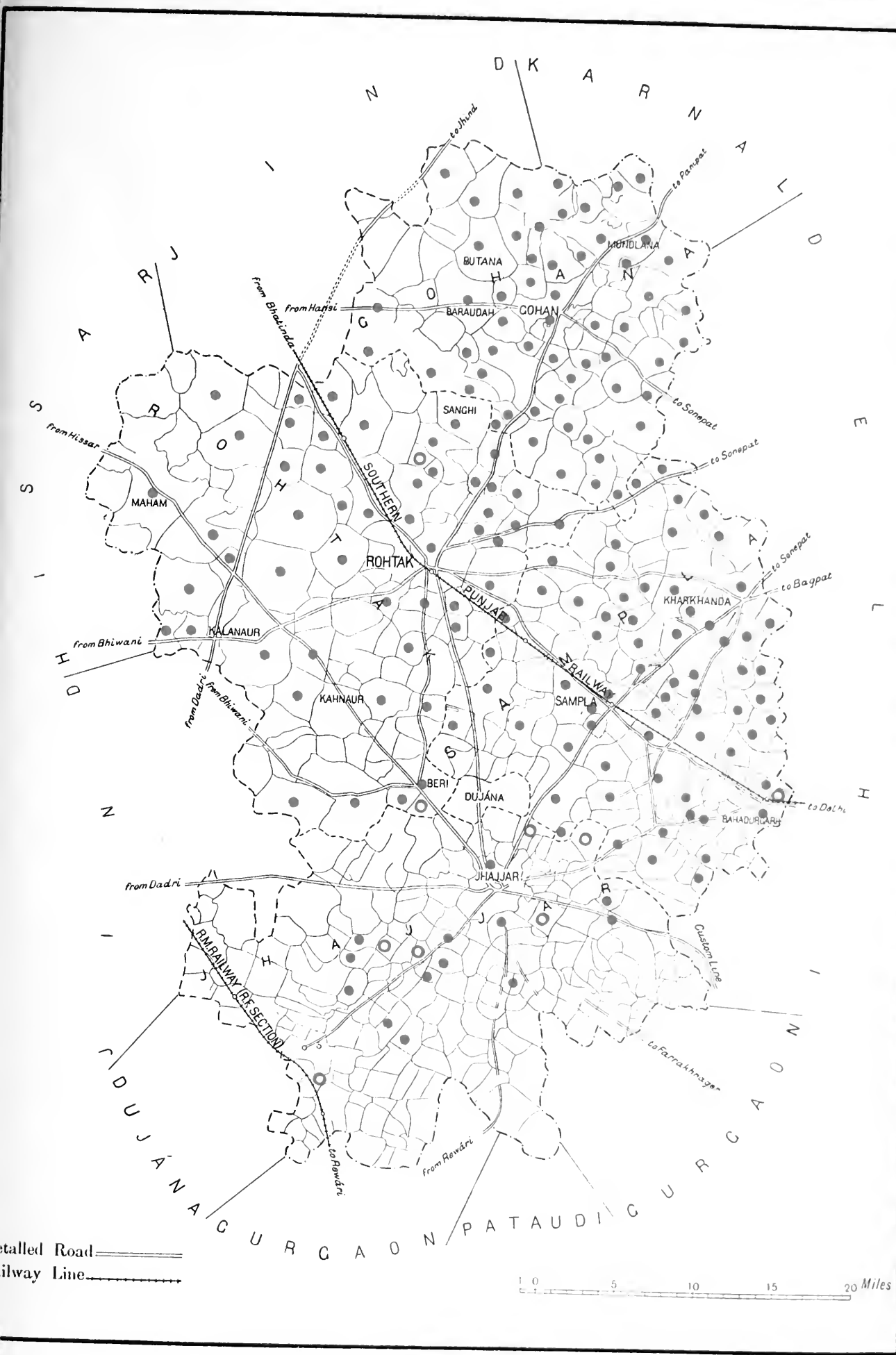




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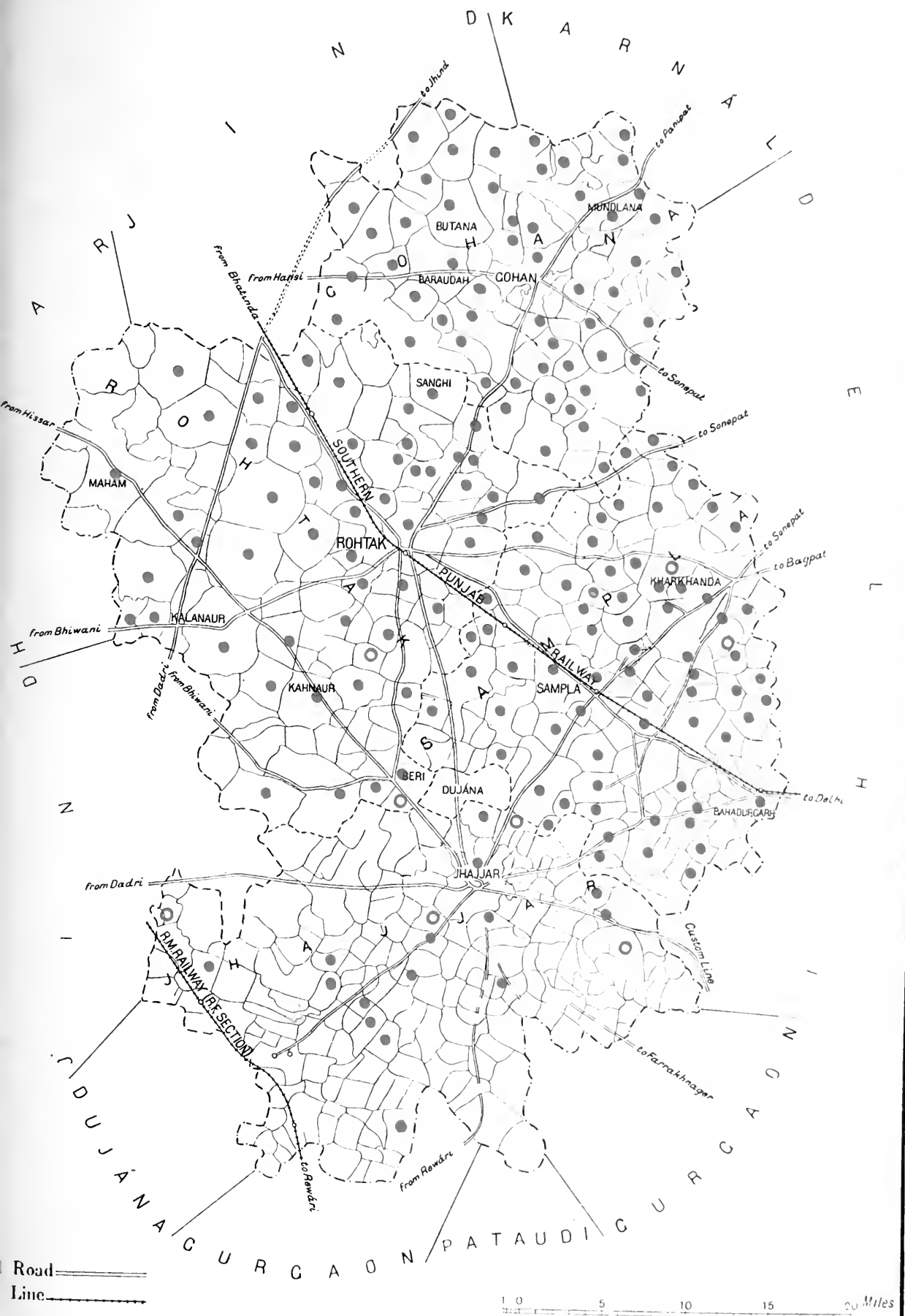
February, 1907





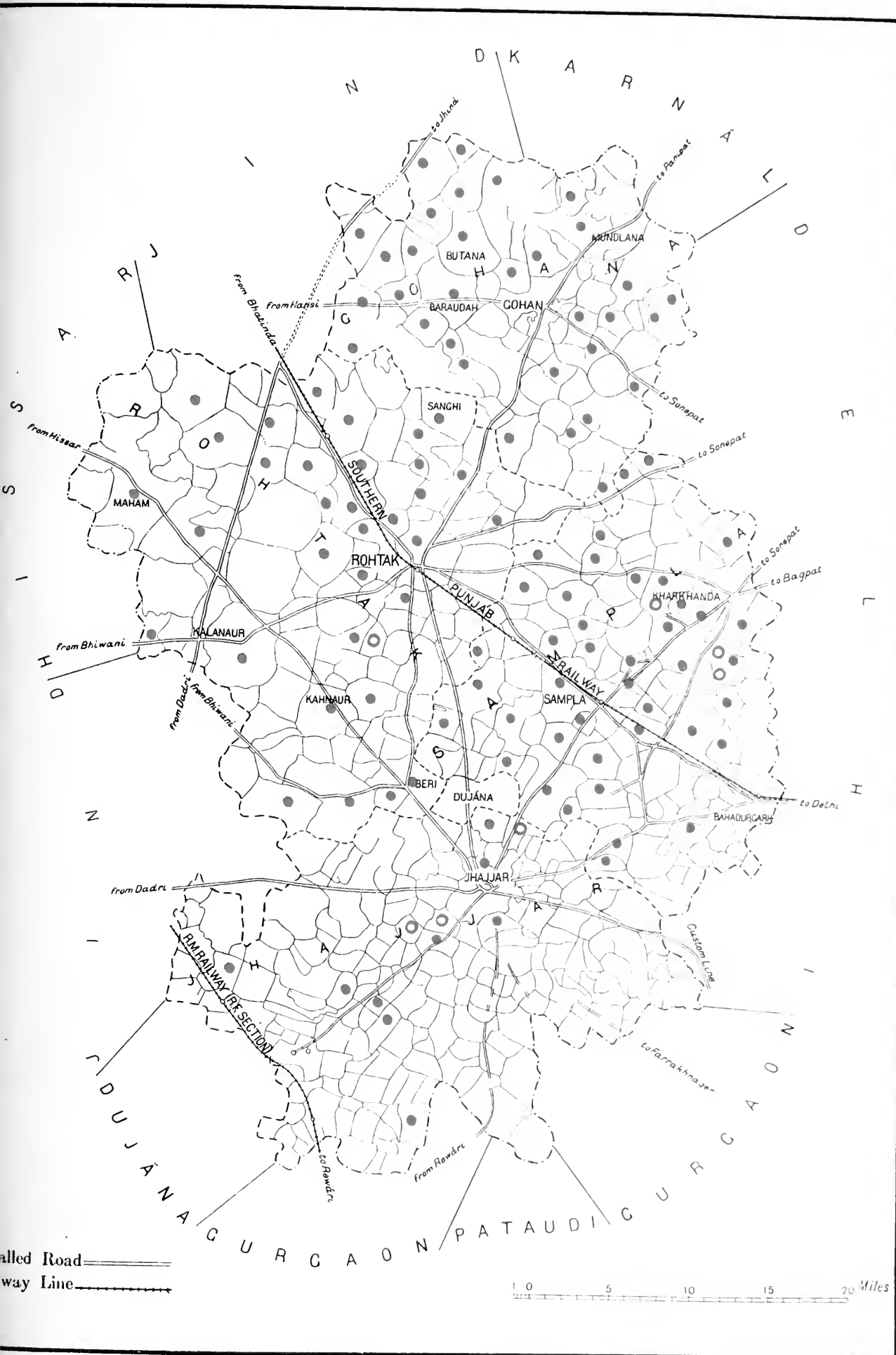
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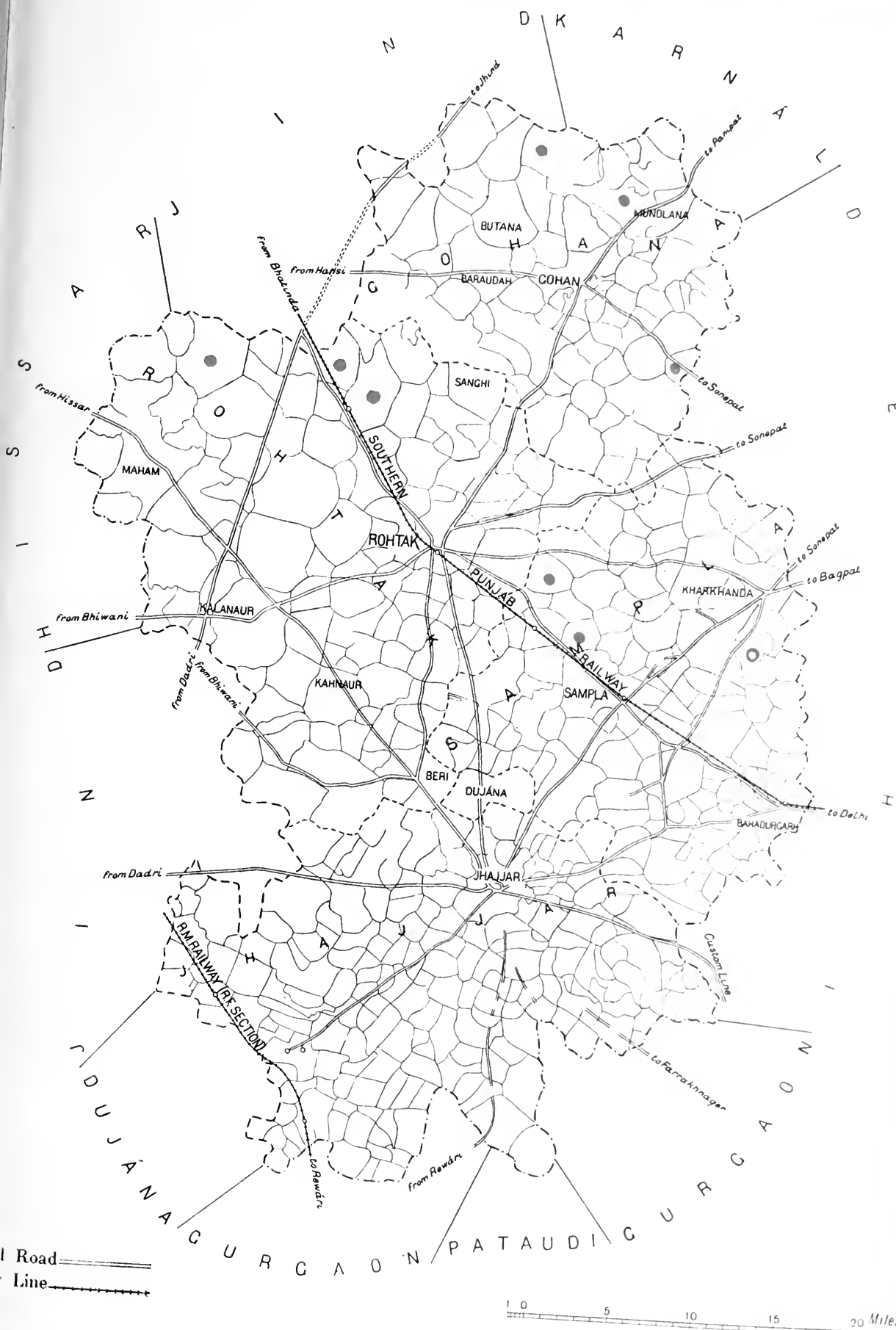
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May, 1907



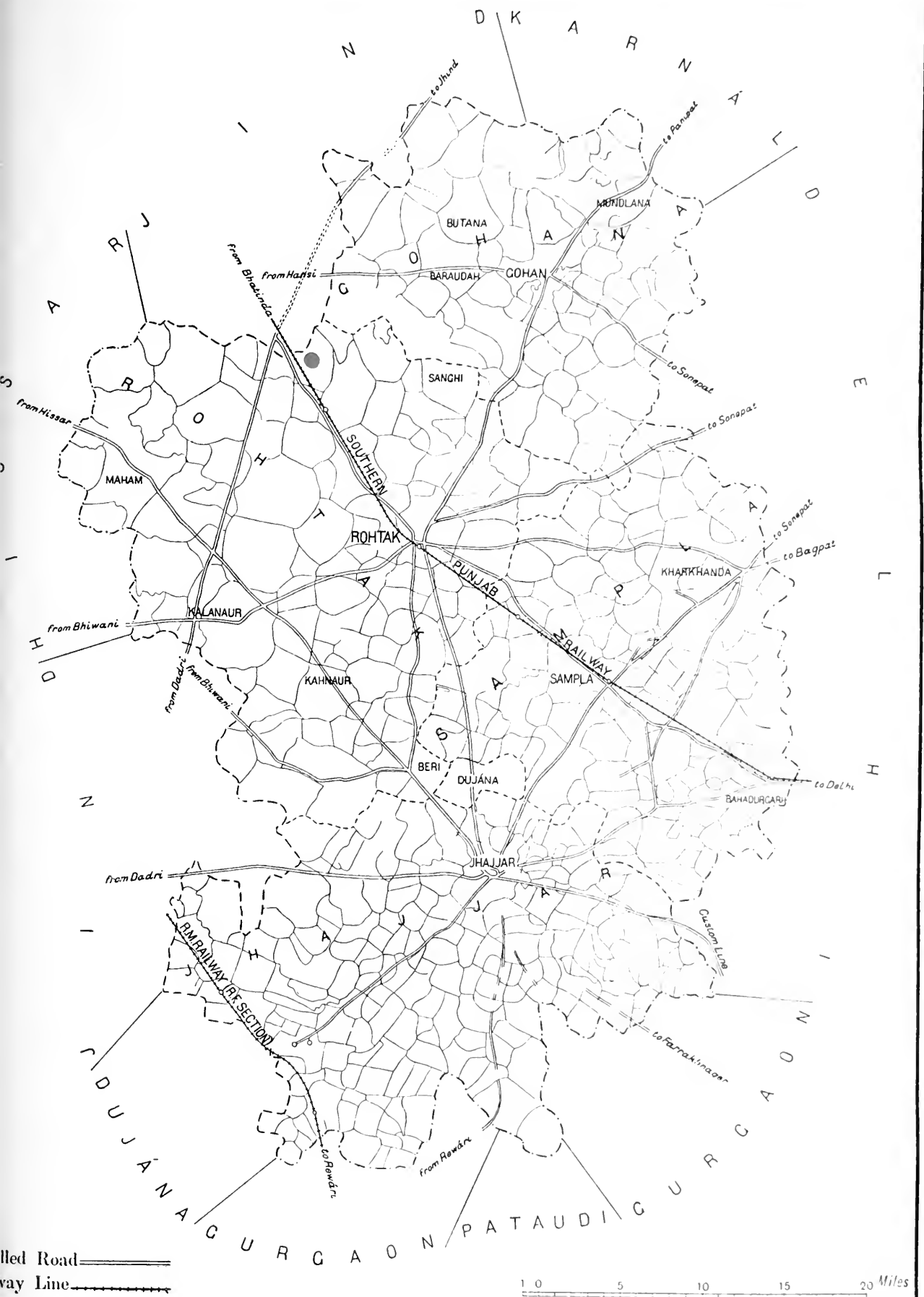
ROHTAK

June 1927



ROHTAK

July, 1907



ROHTAK

August, 1907



The bridge over between this epidemic and that of 1903—04 is therefore clear and easily traceable. The patterns of the maps, taking into consideration the facts that the epidemic was a severe one and that it had its origin probably from several centres left over from the previous epidemic, do not differ from those of the first outbreaks. They even suggest a spread out from the centres which showed infection early in the epidemic.

(d) *Epidemic of 1905—06.* At the end of the epidemic of 1904—05, namely, in May and June 1905, infected villages were plentiful and were scattered over practically the whole district. It is not surprising, therefore, to find the present outbreak springing up in places widely separated from each other and also in villages which had not been infected in 1904—05. This epidemic was a small one, only 69 villages being infected. Taking into account that the infection was probably carried over from the epidemic of 1904—05 in several centres the patterns of the maps in no way differ from those of the first two epidemics.

(e) *Epidemic of 1906—07.* One death in each of the months of July and August 1906 was reported from the town of Kandla in the south-west corner of the district. This town had returned many deaths as late as May 1906. The epidemic proper, however, began in several centres at a distance from one another (vide October and November maps). The patterns of the maps suggest, as in the first epidemics, a spread out in the immediate neighbourhood of these centres and at the same time a spread to villages at a distance. The important thing to note is that the patterns are in no way different from those of the maps of the early epidemics, in which, as we have remarked, importation may be considered to have been the cause of the origin of the outbreaks in the villages.

C. *Amritsar District.*

(a) *Epidemic of 1901—02.* This epidemic, which was a small one, began in Amritsar town in February 1902.

The patterns of the map do not suggest at the beginning a regular centrifugal spread from Amritsar town. During April and May there was, however, a marked increase around Amritsar and also around some of the villages which were infected in March.

It is worthy of note that the northern tehsil of Ajnala remained practically free during the whole of this epidemic.

(b) *Epidemic of 1902—03.* Amritsar City was the only place which reported plague deaths throughout the off season. The patterns of the maps suggest a spread out from Amritsar itself and from the places which first reported deaths in October 1902.

Infected villages were at first confined to the centre of the district and only later on in the epidemic did the outlying north-west and south-east portions become infected. As importation must have been the all-important factor in the spread of this epidemic the patterns of the maps should be carefully studied. 32 villages were still reporting deaths as late as July 1903.

(c) *Epidemic of 1903—04.* The place in which the infection remained over from the epidemic of 1902—03 to that of 1903—04 cannot be clearly demonstrated. Plague deaths were reported in Amritsar City till the end of August 1903 and appeared again in November just to the north of this City. There were two months during which no plague deaths were reported.

The patterns of the maps suggest a spread out from centres established by January 1904. They resemble those of the previous epidemic.

(d) *Epidemic of 1904—05.* In August 1904 a village in the north of Amritsar tehsil returned two deaths. It had not been infected during the epidemic of 1903—04, the last death having occurred 13 months previously. In September four more villages returned deaths. Three of these villages had never been infected before while the fourth had enjoyed a free interval of 15 months. It seems likely that into every one of these villages the infection was imported either from one of the others or from places infected at the end of the previous epidemic.

The patterns of the maps in no way differ from those of the previous epidemics. They suggest a spread out from villages which became infected early in the epidemic, namely, by October 1904.

(e) *Epidemic of 1905—06.* Amritsar town had no interval of freedom between the epidemic of 1904—05 and that of 1905—06. It was the only place which reported deaths during the months of August, September and October 1905. The patterns of the subsequent maps strongly suggest a spread out from three centres, namely, Amritsar town and centres on the north-east and east of Amritsar tehsil.

(f) *Epidemic of 1906—07.* Amritsar City continued to report deaths up till 25 August, 1906, bridging over a considerable portion of the off season. It was the only place which returned deaths in August.

After this date no more deaths occurred in Amritsar town until January 1907.

The epidemic proper began in two centres, one in the north-east of the district in Ajnala tehsil and the other in the east corner. It was well advanced before Amritsar City began to return deaths. The patterns of the maps suggest a spread out from the places infected in the early months of the epidemic and in no way differ from those of previous epidemics.

D. *Summary.*

In the three districts of Rohtak, Mozuffarnagar and Amritsar the methods of spread from village to village or from four to six consecutive epidemics, beginning with the first one, have been traced on monthly maps.

The patterns of the maps of the later epidemics are of the same type as those of the first two epidemics, both of which may be presumed to have owed their spread to importation and not to recrudescence. They, as a rule, suggest a gradual spreading out from centres or foci which became established early in the epidemic. These centres of origin may not be the same in different years. In Amritsar district in some of the epidemics Amritsar City seemed to be the centre from which the infection spread, while in others it was not infected till late on in the outbreak.

I. PREVIOUS PLAGUE HISTORY OF THE VILLAGES INFECTED IN EACH EPIDEMIC.

We propose now to analyse in each district the previous plague history of the villages infected in each epidemic with the object of obtaining data regarding the relative proportion of those which had never been infected, of those which had been infected the epidemic previous, and of those which had escaped for either one or more epidemics.

The analysis of the data available is contained in Tables VIII—X. These tables require little or no explanation and all lead to the same conclusions.

In the case of the first two epidemics, as 85 to 100 p.c. of the villages infected had never before suffered from plague, importation must have been the chief factor in the origin of the outbreak

in each village. In the case of the epidemics either small or large following on severe ones, *e.g.* the third epidemic in Rohtak district, the fourth epidemic in Mozuffarnagar district and the third, fourth, and fifth, epidemics in Amritsar district, from 50 to 85 p.c. of the villages had been infected in the previous year. The present data in these instances, therefore, do not allow us to come to any definite conclusion as regards the relative importance of importation and recrudescence.

In all the other epidemics, however, that is to say, in those years in which the previous epidemic was slight or moderate in extent, the percentage of villages which had shown infection during any period less than 18 months is so small that unless it is possible for the infection to lie dormant over at least one epidemic season, that is to say for 18 months or longer, we must conclude that importation played the most important part in the spread of these outbreaks¹. In addition to the data collated in these tables, we have to consider that importation is at least as likely to occur into villages which have been previously infected as into villages which have never been infected.

IV. PREVIOUS PLAGUE HISTORY OF VILLAGES INFECTED AT THE BEGINNING OF EACH EPIDEMIC.

We have already in passing drawn attention to the fact in that certain instances villages, which began to return plague deaths at the very beginning of an epidemic season, had been infected late in the previous epidemic with perhaps no interval at all or one of only two months free from deaths. This observation suggests that in these villages the infection survived probably as acute plague in the rat. We propose now to consider more fully for each district the data referring to this question, taking the villages first infected in each epidemic and inquiring into their previous plague history. We shall by this means obtain an idea of the proportion of villages reporting deaths early in an epidemic which probably harboured the infection from one epidemic to another as acute rat plague. These data are contained for Rohtak district in Tables XI—XV, for Mozuffarnagar district in Tables XVI—XX, and for Amritsar district in Tables XXI—XXVI. Tables XV, XX, and XXVI show for each district the data referring to villages in which

¹ It is possible that a rat epidemic might occur without a human epidemic. Owing to the intimate relation between men and rats in these villages, this is very unlikely: the conditions elsewhere are quite different.

the infection was very probably carried over from one epidemic to another as acute rat plague.

The following remarks on these several tables will lead to their better understanding.

A. Rohtak District.

(a) *Epidemic of 1903—04.* (Table XI.) As this was the first introduction of plague into the district none of these villages had ever been infected before. It is, however, interesting to note that between November 1903 and February 1904 ten villages had returned plague deaths.

(b) *Epidemic of 1904—05.* (Table XII.) This was a very severe epidemic, following on one in which only a small proportion of the villages had been infected.

From August to November 1904 inclusive 15 villages returned plague deaths and of these nine had been infected during the previous epidemic. When we consider that only 57 villages in the whole district had been infected in 1903—04, this high proportion is strong evidence of the infection having been carried on in some at least of these villages. This presumption is supported from the fact that the interval during which no plague deaths were returned from some of the villages was a comparatively short one. Thus out of the nine villages three had a free interval of only two months, two of three months, two of four months and two of five and six months each.

In December six new villages reported deaths: none of these had been previously infected.

(c) *Epidemic of 1905—06.* (Table XIII.) This epidemic it will be remembered followed a very severe and widespread outbreak but was itself mild and limited in extent, only 30 villages returning deaths.

In October 1905 two villages reported deaths. Both of these villages had been infected in the previous epidemic and had been free from deaths for three or four months respectively.

In December and January four other villages began to return deaths. These four villages had also been infected in 1903—04 but had enjoyed a free interval of from five to seven months.

(d) *Epidemic of 1906—07.* (Table XIV.) In the epidemic of 1906—07 half the villages of the district were infected, a contrast to the previous one in which only 30 villages reported deaths. During August and September already four villages were returning deaths.

Two of these villages had been infected in the previous epidemic and had enjoyed only two months in which no deaths occurred. The other two villages had not been infected since 1904—05 and in both instances there were 15 months during which no plague deaths had been reported.

During October, November and December, several more villages returned deaths. Of these only two had been infected in the epidemic of 1905—06 and the interval of apparent freedom was three and five months respectively.

Thus during the early months of this outbreak out of 11 villages which reported deaths only four had been infected in the previous epidemic, the intervals of freedom being in two instances two months, in a third three months, and five months in the fourth. In the case of the other seven villages no plague deaths had been reported for from 15 to 21 months.

B. *Mozuffarnagar District.*

(a) *Epidemic of 1903—04.* (Table XVI.) During September 1903 to January 1904 inclusive 16 villages began to report deaths from plague. Of these villages 13 had not been infected before, and three had suffered during the previous epidemic; one of these, however, had returned only one death in the epidemic of 1902—03.

In the case of both the other two villages there was an interval of three months in which no plague deaths were reported. It is also important to note that both these villages began to report cases very early in the epidemic, namely, in October 1903.

(b) *Epidemic of 1904—05.* (Table XVII.) The epidemic of 1904—05 was a severe one following on a comparatively mild one.

During the first four months (July—October) plague deaths occurred in 26 villages. In two of these villages the bridge over was complete, that is to say, there was no interval during which the villages were free from deaths between the beginning of this and the end of the last epidemic. One village was infected from April to September and the other from May to October inclusive. A third village, which returned one death in August but after this date none till October, had been badly infected as late as June. It is probable, therefore, that the infection was present as acute rat plague during this period, namely, June—October. A fourth village had an interval of two months, July and August, free from plague deaths. In the case of

ther four villages which evidently had comparatively short intervals of freedom, three to five months, it is doubtful whether they were really infected or not in the outbreak of 1903—04, as each then returned only one or two deaths.

Of the remaining 18 villages, which began to return plague deaths early in this epidemic, 15 had never been infected before, and the other three had enjoyed a free interval of from 17 to 18 months, that is to say, had not been infected during the previous epidemic.

From this short account it is seen that only in a very few of the villages infected at the beginning of this epidemic was it reasonable to suppose that the origin of the disease was due to recrudescence from remnants left over from the previous epidemic. In these few villages, however, and especially in the two which had no free interval, it is likely that the infection persisted throughout the off season in an acute form amongst the rats, constituting a complete bridging over of one epidemic to the other.

(c) *Epidemic of 1905—06.* (Table XVIII.) This was a very small outbreak following on a very severe one.

We have analysed the previous plague history of 19 villages which began to report deaths at the beginning of the epidemic, that is to say, during August 05—February 06. Of these villages six had never been infected before; one had been infected in the 1903—04 epidemic and had enjoyed an interval of freedom for 19 months; the remaining 12 villages had been infected during the previous epidemic. But in the case of four of these villages there was only one or two deaths reported in the epidemic of 1904—05, and in three others there was only one death in the epidemic of 1905—06, so that we are justified in concluding that in one of these seven villages was it likely that the infection had survived from one epidemic to the other. That leaves us with five villages which were definitely infected in both epidemics. The intervals during which no deaths were reported in these villages was 2, 5, 7, 11 and 12 months respectively.

(d) *Epidemic of 1906—07.* (Table XIX.) This was a very severe epidemic, affecting as it did more than half the villages of the district. It will be remembered that it followed on a very mild outbreak.

We have traced the previous plague history of the 45 villages which were the first to report deaths, that is during the period from July to December 1906 inclusive.

Twelve of these villages had never been infected in any previous epidemics; two had not returned any deaths since the epidemic of

1903—04, having enjoyed a free interval of 29 and 30 months respectively : twenty had been last infected in the severe outbreak of 1904—05, having escaped in the epidemic of 1905—06. The interval of freedom from deaths in these villages varied from 17 to 22 months. In none of these villages therefore, is it at all likely that the infection survived from one epidemic to the other. We are now left to analyse the data in the case of the remaining 11 villages which were infected in the two consecutive epidemics of 1905—06, and 1906—07. From the table it will be seen that in the case of three of these villages there was only one death in one or other of the epidemics so that the infection was probably not indigenous. In the remaining eight villages the interval during which no deaths were reported varied from one to six months, and in all these, there is the possibility of the infection bridging over the epidemics as acute plague in the rat. Amongst these villages Kandla, a town with a population of over 11,000, has a most interesting history. It was infected badly in the epidemic of 1905—06, the last death being reported on 25th May, 1906. In June no deaths were reported, but in each of the months of July and August one death came to light. During September and October again no deaths were returned as due to plague, but in November between the 8th and 14th seven deaths were reported as plague. From this latter date until the 24th February, 1907, the epidemic appeared to be entirely in abeyance. It then started again and between 24th February and 4th July, 1907, 600 deaths took place.

Such a history as this appears to us strongly in favour of the supposition that in this town acute plague amongst the rats accompanied by a few human cases was present during the whole off season and bridged over the interval between the two epidemics.

C. *Amritsar District.*

(a) *Epidemic of 1902—03.* (Table XXI.) Amritsar City had no interval free from deaths between the epidemic of 1901—02 and that of 1902—03. In July 1902 there were three, in August 14 and in September 47 deaths. It is certain, therefore, that acute rat plague was present in the city throughout the off season.

Of the 45 villages which began to report deaths early in this epidemic, namely, in October and November, only seven had been previously infected. They had been free from deaths for from three to four months. It is probable, therefore, that in some of these villages also acute rat plague bridged over the two epidemics.

(b) *Epidemic of 1903—04.* (Table XXII.) In Amritsar City the epidemic of 1902—03 was continued into August, the last death being reported on 26. VIII. 03. No more deaths were returned until January 1904 so that the bridge over of the two epidemics as acute rat plague cannot be completely demonstrated.

Up to the end of December 1903 only two villages returned plague deaths. One of these had reported a single death twelve months before while the other had enjoyed a free interval for six months.

During January 1904, 20 villages began to return deaths. Three of these had never been infected before, one had been last infected in the epidemic of 1901—02, while the remainder had reported deaths in the previous epidemic. The shortest interval of freedom, however, which any of these villages enjoyed was six months, while some of them had been free for as long as 10 to 11 months.

(c) *Epidemic of 1904—05.* (Table XXIII.) In August and September 1904 five villages began to return plague deaths. Three of them had never been infected before, while the other two had had free intervals of 13 and 15 months respectively. In not one of these villages, therefore, is it probable that the epidemics were bridged over by acute plague in the rat.

During October and November, 1904, 37 villages became infected. Of these villages eight had never been infected in any of the previous epidemics and 13 had been for 14 months or longer free from deaths. That leaves us with 16 villages which had reported plague deaths in the previous epidemic of 1903—04. Three of these villages had a free interval of three months, seven had four months, five had five months and one six months.

(d) *Epidemic of 1905—06.* (Table XXIV.) Amritsar City was still reporting plague deaths in August 1905; no free interval occurred between the end of the last epidemic and the beginning of that of 1905—06. From the beginning of August till the end of the year in this city 40 deaths were reported as being due to plague. There can be no doubt, therefore, that acute rat plague must have been present in Amritsar City throughout the whole of the off season of 1905.

During August, September and October 1905, no other place except Amritsar City reported deaths. During November and December 1905, three more villages were recognised as being infected. Only one of these had been infected in the previous epidemic, the last death having been reported in March 1905, *i.e.* there was a free interval of at least seven months.

During January and February 1906, 22 villages began to report plague deaths. As will be seen from the Table everyone of these villages had been infected in some previous epidemic, 19 in the severe epidemic of 1904—05 and the other three in that of 1903—04. The shortest interval of freedom from deaths enjoyed by any of the villages infected in 1904—05 was five months and the longest 10 months.

(e) *Epidemic of 1906—07.* (Table XXV.) In August 1906 Amritsar City still continued to report deaths from plague. The last death occurred on 25. VIII. 06, and from that date till January 1907 the city was apparently free from the disease, that is to say, no deaths were returned.

In September two villages began to report. One of these villages had not been infected since December 1904, while the other had only been two months free from deaths.

In October three more villages were reported to be infected. One of these villages had never been infected before while the other two had been free from deaths for 15 and 40 months respectively.

During November and December 1906 38 new villages began to return plague deaths. Of these villages three had never been infected before, 23 had been infected in some previous epidemic but not in that of 1905—06, while the remaining 12 had returned deaths during the last epidemic, the interval of freedom enjoyed being from 4—6 months.

Lastly in Tables XV, XX and XXVI we have for each district collected together from the various epidemics the data referring to those villages in which, judging from the short period of freedom from plague deaths, the infection was probably carried over from one epidemic to the next one as acute rat plague. It would appear then that at the beginning of each epidemic amongst the first villages to report plague deaths are some in which deaths have occurred late in the previous epidemic and that there are instances in which there may be no interval of freedom at all. It is to be remembered that the data under analysis only refer to plague deaths not to cases which recover, and that it is more than likely that both cases and deaths occurring in small number in the off season would be concealed, the deaths being returned as due to some other cause. It is of course possible that the epizootic amongst the rats might continue without any plague cases in the human population, held in check by the unfavourable conditions to which we have drawn attention in another paper (*Journal of Hygiene*, vol. VIII. p. 266), namely, a mean temperature above 86° F. and a paucity of rat fleas. If, there-

fore, all human plague cases had come within our ken and especially if the rat population could have been examined as was done in Bombay it is probable that the bridging over the off season by acute plague in the rat would have been more clearly demonstrated and in more villages than we have been able to do with the data at our disposal.

Two points of interest and importance require mention before leaving this part of our subject. First, it is seen from the tables that the villages in which the epidemics are bridged over are of large size with a population considerably greater than that of the average village of the district. It is also seen that with few exceptions, *e.g.* Amritsar town, the villages in which apparently the infection survives the off season vary from year to year.

D. *Summary.*

Villages which return plague deaths early in an epidemic are in some instances those which were infected late in the previous epidemic. There may be no interval free from deaths or it may be as short as one to three months. In the great majority, however, of early infected villages the interval of freedom is very much longer, suggesting a fresh importation of the infection.

The villages in which one epidemic is bridged over from the previous one are as a rule of large size. They also, with the exception of the large towns such as Amritsar, vary from year to year.

V. FUTURE PLAGUE HISTORY OF THE VILLAGES INFECTED IN THE COURSE OF THE Milder EPIDEMICS.

In all three districts some of the epidemics were very mild, affecting only a comparatively small number of the total villages. It was a simple matter, therefore, to follow up the history of all these villages and to put into tabular form the data obtained. This has been done for two epidemics in two of the districts, namely Rohtak and Mozuffarnagar.

An analysis of these data throws light on the question of the bridging over of the epidemics. The data and their analysis are contained in the following tables:—

- (1) Rohtak district—Tables XXVII—XXXII.
- (2) Mozuffarnagar district—Tables XXXIII—XXXVIII.

To these tables the following remarks apply.

A. Rohtak District.

(a) *Epidemic of 1903—04.* From Table XXVII it is seen that of the 57 villages which were infected during this epidemic six were not again infected and seven did not return any more deaths until the epidemic of 1906—07, that is to say, nearly two years afterwards. Further 13 villages reported less than three deaths during the epidemic under consideration, suggesting the probability that they were not truly infected but that the cases contracted their infection elsewhere. We are, therefore, left with 31 villages, which, infected in the epidemic of 1903—04, were again infected in 1904—05. It is possible, therefore, that in every one of these villages the second epidemic had its origin in remnants left over from the first one. When, however, we consider that more than half the villages of the district were infected in 1904—05 and that the great majority of them presumably owed the origin of infection to importation, it is justifiable to assume that a certain proportion of these 31 villages were also infected by importation. This assumption receives material support from an analysis of the data, which refer to the period during the second epidemic at which these villages first returned deaths (Table XXVIII) and which show the number of months during which they were free from plague deaths (Table XXIX).

From Table XXVIII it is seen that in only nine out of the 31 villages were plague deaths returned at all early in the epidemic of 1904—05, that is to say, before January 1905. Further, from Table XXIX it is seen that only in the case of seven of the villages was the free interval less than five months.

(b) *Epidemic of 1905—06.* We have analysed the future history of the villages infected during this epidemic in the same manner as was done with those of the 1903—04 epidemic. The data are set forth in Table XXX.

In all 30 villages returned plague deaths; four of these were not infected in the epidemic of 1906—07 and six had less than five deaths, and these we have taken to be imported cases, that is to say, received their infection elsewhere. We are, therefore, left with 20 villages which were definitely infected in both epidemics and in the case of all of which it might be argued that the infection in 1906—07 originated from remnants left over from 1905—06. But as happened in the epidemics

of 1903—04 and 1904—05, very few, namely, four, of these 20 villages returned deaths early in the second epidemic (Table XXXI) and the interval free from deaths was five months or less only in the case of four of the villages (Table XXXII).

B. *Mozuffarnagar District.*

(a) *Epidemic of 1902—03.* In Table XXXIII are set forth the details as regards plague infection of the 25 villages which reported plague deaths during this epidemic. From this table it is seen that 11 of the villages returned only one or at most two deaths, so that it is more than probable that these cases were imported and that there was no indigenous plague. They are, therefore, left out of account. Of the remaining 14 villages three were never again infected. The analysis of the data as regards the future plague history of the remaining 11 villages is set forth in Tables XXXIV and XXXV. Six of the villages were again infected in the next epidemic but only two of these six returned cases at all early in the course of the outbreak, having enjoyed a free interval of three months or less. The other four became infected well on in the epidemic at a time when many other villages were reporting deaths. We are left with five villages which, having escaped in the epidemic of 1903—04, were infected in some subsequent outbreak. They enjoyed free intervals of from 16 to 20 months.

(b) *Epidemic of 1905—06.* This was a more widespread epidemic than that of 1902—03, 69 villages in all reporting plague deaths. It was followed by a very severe outbreak, the last of which we take any cognizance.

The data concerning the infected villages are set forth on Table XXXVI. From an analysis of this table it is found that seven of the villages were not infected in the following epidemic leaving 62 which reported deaths in both epidemics. But in the case of nine of these villages there was only one death reported in one or other of the epidemics, so that we are left with 53 villages which can be said to have been definitely infected in both epidemics. Further analysis shows us that the great majority of these villages began to return deaths well on in the epidemic, when a great many other villages were already infected and that only a small proportion had a short interval of freedom or reported deaths early in the epidemic (Tables XXXVII and XXXVIII).

C. *Summary.*

A relatively small number of villages in the Rohtak district were infected in the epidemics of 1903—04 and 1905—06, and in the Mozuffarnagar district in the outbreaks of 1902—03 and 1905—06. Even when the majority of the villages which returned deaths in these epidemics were again infected in the following epidemic, analysis of the data shows that in the great majority of these villages the infection did not take place until late in the epidemic at a time when many other villages, not infected in the mild epidemic, were returning deaths. Importation, therefore, is at least equally likely to have been the origin of the second outbreak as recrudescence.

VI. FUTURE PLAGUE HISTORY OF VILLAGES INFECTED AT THE END OF EACH EPIDEMIC.

We have already seen that the villages infected at the beginning of an epidemic are sometimes, but comparatively rarely, those which have returned deaths at the end of the previous epidemic and that in some instances there may be no period of freedom from plague deaths, or that the free interval may be as short as from one to three months. We propose in this section to inquire into the future plague history of those villages which reported deaths at the end of the various epidemics in the different districts. We hope thus to obtain some idea of the proportion of late infected villages which are infected early in the next epidemic. The importance, also, of such an inquiry from a prophylactic point of view is evident. The crude data as regards the villages in Rohtak district are set forth in Table XXXIX, in Mozuffarnagar district in Table XL, and in Amritsar district in Table XLI. The analysis of these data is contained in Tables XLII—XLIV.

A. *Rohtak District.*

(a) *Villages infected at end of the epidemic of 1903—04.* In June 1904 nine villages had their last plague death, while in July the infection apparently remained only in one.

From all of these ten villages deaths were reported in the next epidemic, which it will be remembered was very severe and widespread. When, however, we come to consider the interval during which these

ten villages returned no deaths (Table XLII) we see that the majority of them did not again show infection till late on in the 1904—05 epidemic, which by that time was widespread and affecting many villages. Only three of the ten villages had a free interval of less than five months.

(b) *Villages infected at end of the epidemic of 1904—05.* This was a severe epidemic and a considerable number of villages still reported deaths towards the end, namely 36 in June and one in July.

What was the plague history of these 37 villages in the following epidemics of which that of 1905—06 was slight and that of 1906—07 was severe? Eight were not infected in either of these epidemics, 20 were infected in 1906—07 but not in 1905—06, leaving only nine which returned deaths in the latter epidemic. In two of these nine villages very few deaths occurred, six or under, which suggests that there may have been no indigenous plague in them.

When we now consider the interval of freedom from deaths which the 29 villages subsequently infected enjoyed we find (Table XLII) that the great majority of them were free for a long period, 19—23 months, and that all the nine villages which returned deaths in the epidemic of 1905—06 were free for at least five months and the majority of them for 8—9 months.

(c) *Villages infected at the end of the epidemic of 1905—06.* Of the villages infected during this mild epidemic only four still reported deaths in June, so that we shall include in our analysis eight others in which the last deaths occurred in May, 12 villages in all. Of these 12 villages three returned no deaths while nine became infected in the epidemic of 1906—07, which it will be remembered was severe and widespread. When we analyse (Table XLII) the data referring to the interval during which these villages returned no plague deaths it is seen that two had a free interval of 2 to 3 months, while the remaining seven villages did not report deaths till late on in the epidemic, when many other villages were already infected. They had enjoyed a free interval of from 7 to 10 months.

B. *Mozuffarnagar District.*

(a) *Villages infected at the end of epidemic of 1902—03.* In the epidemic of 1902—03, 11 villages still returned deaths in May or June 1903. Of these one was not again infected, while the period of freedom enjoyed by the other ten varied from 3 to 20 months. From Table

XLIII it will be seen that the great majority did not again report deaths until after an interval of more than eight months.

(b) *Villages infected at the end of epidemic of 1903—04.* In the epidemic of 1903—04, 22 villages were still returning plague deaths in June 1904. Two of these had no free interval, deaths continuing to occur right through the off season; two others had an interval of freedom of one and two months respectively; further, 10 more villages returned deaths in the epidemic of 1904—05, but did not begin until late on in the epidemic, at a time when many other villages were infected. The remaining eight villages were not infected till a subsequent epidemic, escaping altogether during the 1904—05 outbreak.

(c) *Villages infected at the end of epidemic of 1904—05.* In the epidemic of 1904—05, 35 villages returned plague deaths as late as June 1905. Of these villages seven were not again infected, 23 did not return deaths till the epidemic of 1906—07, while the remaining five were infected in the epidemic of 1905—06 but not until late on in the course of the outbreak.

(d) *Villages infected at the end of epidemic of 1905—06.* In the epidemic of 1905—06, which it will be remembered was a very mild one, only six villages reported deaths in June. All these were again infected during the severe epidemic of 1906—07, but deaths did not begin to occur till the epidemic was fairly well advanced, the interval of freedom enjoyed being from 4 to 8 months.

C. *Amritsar District.*

It is unnecessary to do anything further than draw attention to the table containing the data (XLI) and that showing the analysis (XLIV). These are of the same nature as those already described for Rohtak and Mozuffarnagar districts.

D. *Summary.*

Villages which return deaths at the end of one epidemic may or may not show infection during the next epidemic. Only in a very few instances is there no interval free from plague deaths between one epidemic and another and in a few more the interval is from 1 to 4 months. On the other hand the great majority of the villages infected at the end of an epidemic do not report deaths early in the subsequent

epidemic. They are either not infected at all or the free interval is from 6 to 10 months. By this time the epidemic is already widespread and many other villages are infected.

VII. THE QUESTION WHETHER PLAGUE TENDS TO RECUR IN VILLAGES IN SUCCESSIVE EPIDEMICS.

As this question is elsewhere fully discussed by Dr Greenwood we need not do more than refer to the tables annexed and to the general conclusions these would appear to us to support.

We would remind our readers that in our study of plague in the Punjab villages of Dhand and Kasel (*Journal of Hygiene*, vol. VII. p. 984) an attempt was made to determine whether houses which were infected in one epidemic were especially liable to be again infected in any subsequent epidemic, and that we arrived at the very definite conclusion that plague showed no tendency to recur in houses during successive epidemics. The same method was now used to ascertain if the villages in the three districts of Rohtak, Mozuffarnagar and Amritsar owed their infection to chance or not.

The data are given for Rohtak in Tables XLV to XLVII, for Mozuffarnagar in Tables XLVIII to L, and for Amritsar in Tables LI to LIII.

From a study of these tables which show a marked lack of correspondence between the actual and calculated figures it is evident that chances of infection were not altogether random, that is to say, that some villages were more liable to be infected than others.

On thinking over the problem it suggested itself to us that the population of the villages might be an important factor in determining whether a village would become infected or not. We have roughly tested the truth of this hypothesis in all three districts, first by comparing the average population of the villages infected in each of the epidemics, it being remembered that the epidemics varied greatly in severity, and secondly by comparing the average population of the villages infected in none of the epidemics, in one epidemic only, in any two, three etc. epidemics.

These data are contained in Tables LIV to LV for Rohtak district, Tables LVI to LVII for Mozuffarnagar district and Tables LVIII to LIX for Amritsar district. These tables all show the same phenomena.

First, it is seen that in the epidemics in which only a small number of villages returned deaths the average population of these villages was

very much greater than that of the villages infected in the years in which a large number reported deaths, that in fact, there was an inverse proportion between the number of villages attacked in an epidemic and the average population of these villages.

Secondly, it is seen that the average population of the villages which have enjoyed complete immunity is very small and that the greater the number of times the villages are infected the greater the average population. There is in fact a direct proportion between the number of epidemics in which villages are infected and the average population of the villages.

Thirdly, in the case of Rohtak there is another piece of evidence which shows that population is an important factor in determining the chances of infection of a village. In an early part of this paper we draw attention to the fact that in the Jhaggar tehsil of Rohtak district there were not only a smaller number of inhabitants but also a larger number of villages than in any of the other three tehsils, the result being that the average population of the villages in this subdivision was comparatively small, much below that of the other tehsils and of the district as a whole. This being so we should expect to find that the villages of the Jhaggar tehsil had suffered less than those of the other tehsils. And this expectation is shown to be correct both from the figures set forth in Table LX and from the maps 1 to 6.

From the table it is seen that, while 51·9 p.c. of the villages in Jhaggar tehsil were never infected with plague, in the other tehsils this percentage was between 7 and 19. Further it is seen that the percentage of villages in Jhaggar infected in two and three epidemics was very much less than in Rohtak, Gohana and Sampla.

In the maps 1 to 5 the villages infected in none of the epidemics, in only one epidemic, in any two, in any three and in all four epidemics respectively have been marked out. It is seen at once that as regards villages infected in all four epidemics there is no marked grouping, that Jhaggar is remarkably free from villages infected in any three epidemics and contains much the greatest number of villages infected in two epidemics and those never infected at all.

SUMMARY.

By comparing the actual number of villages infected in none of the epidemics, in only one, in any two etc. epidemics with the number calculated on the assumption that all villages are equally liable to become infected, it is evident from the lack of correspondence between the actual and calculated figures that the assumption is not correct, that, in fact, some villages are more liable to become infected than others.

On further investigation it would appear that one factor at least which determines this greater liability to infection is the number of inhabitants, the larger villages being more often infected than the smaller.

TABLE I.

Showing the distribution of the population in the four Tehsils of the Rohtak District.

Tehsil	No. of villages	Population	Average population per village
Gohana	79	140,682	1781
Rohtak	109	195,423	1793
Sampla	124	160,262	1292
Jhaggar	187	124,455	665

TABLE II.

Showing a summary of the plague history of Rohtak District for each epidemic.

Epidemic of	Number of villages which returned deaths	Total mortality about
1903—04	37	2500
1904—05	285	27,000
1905—06	30	2000
1906—07	249	30,000

TABLE III.

*Showing the number of villages infected month by month in each
Tehsil of the Rohtak District.*

Month	Year	Rohtak	Gohana	Sampla	Jhaggar	Total
January	1904	3	0	0	1	4
	1905	26	7	14	16	63
	1906	2	3	1	0	6
	1907	4	11	6	0	21
February	1904	9	0	1	5	15
	1905	36	9	34	22	101
	1906	3	5	1	0	9
	1907	10	25	13	1	49
March	1904	15	0	5	11	31
	1905	51	18	51	36	156
	1906	3	8	0	0	11
	1907	33	41	52	6	132
April	1904	15	1	8	13	37
	1905	65	46	68	53	232
	1906	8	11	1	2	22
	1907	52	57	68	19	196
May	1904	15	1	6	13	35
	1905	55	50	65	38	208
	1906	8	11	0	1	20
	1907	55	55	70	19	199
June	1904	7	1	2	2	12
	1905	7	22	33	7	69
	1906	0	4	0	0	4
	1907	34	19	44	13	110
July	1904	1	0	0	0	1
	1905	0	0	1	0	1
	1906	0	0	0	0	0
	1907	3	3	3	0	9
August	1904	0	0	1	1	2
	1905	0	0	0	0	0
	1906	1	0	0	0	1
	1907	1	0	0	0	1
September	1904	0	1	3	2	6
	1905	0	0	0	0	0
	1906	1	2	1	0	4
October	1904	2	1	3	3	9
	1905	0	1	1	0	2
	1906	1	4	2	0	7
November	1903	3	0	0	0	3
	1904	5	1	3	8	17
	1905	0	1	1	0	2
	1906	1	6	2	0	9
December	1903	4	0	0	0	4
	1904	7	4	5	7	23
	1905	0	2	1	0	3
	1906	1	8	2	0	11

TABLE IV

Showing the data referring to the five epidemics in Mozuffarnagar District.

Number and year of epidemic		Number of villages which returned deaths	Total mortality about
1st	1902—3	25	1256
2nd	1903—4	130	8777
3rd	1904—5	313	11,867
4th	1905—6	69	2962
5th	1906—7	579	34,933

TABLE V.

Showing month by month the number of villages which reported plague deaths in Mozuffarnagar District.

Year	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
1902	1	0	0	1	0	0	0	0	0	0	5	2
1903	2	5	9	15	11	3	0	0	1	2	5	7
1904	11	30	51	91	52	22	4	7	12	19	36	64
1905	83	100	163	173	150	35	0	1	1	1	5	7
1906	7	12	29	46	33	6	1	1	1	7	16	35
1907	77	159	288	438	427	169	7	2	2	5	6	10

TABLE VI.

Showing data referring to the six epidemics in the Amritsar District.

Number and year of epidemic		Number of villages which returned deaths	Total mortality about
1st	1901—02	62	2509
2nd	1902—03	506	26,181
3rd	1903—04	445	22,437
4th	1904—05	669	29,930
5th	1905—06	276	8535
6th	1906—07	604	24,503

TABLE VII.

Showing month by month the number of villages which reported plague deaths in Amritsar District.

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1902	0	2	8	23	30	24	7	1	1	4	46	82
1903	118	180	153	299	214	189	32	1	0	0	2	2
1904	24	47	116	329	410	117	14	1	4	17	37	93
1905	178	285	389	461	456	245	39	1	1	1	4	4
1906	14	22	62	158	222	75	11	1	2	5	20	39
1907	59	118	211	411	480	354	69	—	—	—	—	—

TABLE VIII. (*Rohtak District.*)

Showing analysis of data referring to previous plague history of villages infected each epidemic.

	Number and year of epi- demic	No. of villages infected	No. and percent- age of villages never before infected	No. and percent- age of villages infected in pre- vious epidemic	No. and percent- age of villages which escaped infection for one epidemic	No. and percent- age of villages which escaped infection for two epidemics
1st	1903—04	57	57 (100 p.c.)	—	—	—
2nd	1904—05	285	241 (85 p.c.)	44 (15 p.c.)	—	—
3rd	1905—06	30	4 (13·3 p.c.)	26 (86·7 p.c.)	—	—
4th	1906—07	249	52 (20·9 p.c.)	26 (10·4 p.c.)	164 (65·9 p.c.)	7 (2·8 p.c.)

TABLE IX. (*Mozuffarnagar District.*)

Showing analysis of data referring to the previous plague history of villages infected each epidemic.

	Number and year of epi- demic	No. of villages infected	No. and percent- age of villages never before infected	No. and percent- age of villages infected in pre- vious epidemic	No. and percent- age of villages which escaped infection during one epidemic	No. and percent- age of villages which escaped infection in two epidemics	No. and percent- age of villages which escaped infection in three epidemics
1st	1902—03	25	25 (100 p.c.)	—	—	—	—
2nd	1903—04	130	121 (93 p.c.)	9 (7 p.c.)	—	—	—
3rd	1904—05	313	220 (70·3 p.c.)	82 (26·2 p.c.)	11 (3 p.c.)	—	—
4th	1905—06	69	24 (34·8 p.c.)	41 (59·4 p.c.)	4 (5·8 p.c.)	—	—
5th	1906—07	579	249 (43 p.c.)	62 (10·7 p.c.)	227 (39·4 p.c.)	39 (6·6 p.c.)	2 (0·3 p.c.)

TABLE X. (*Amritsar District.*)

Showing analysis of data referring to the previous plague history of villages infected each epidemic.

	Number and year of epidemic	Total no. of villages infected	No. and percentage of villages never before infected	No. and percentage of villages infected in previous epidemic	No. and percentage of villages which escaped infection in one epidemic only	No. and percentage of villages which escaped infection in two epidemics	No. and percentage of villages which escaped infection in three epidemics	No. and percentage of villages which escaped infection in four epidemics
1st	1901—02	62	62 (100 p.c.)	—	—	—	—	—
2nd	1902—03	506	462 (91·3 p.c.)	44 (8·7 p.c.)	—	—	—	—
3rd	1903—04	445	149 (33·5 p.c.)	286 (64·2 p.c.)	10 (2·3 p.c.)	—	—	—
4th	1904—05	669	166 (24·8 p.c.)	345 (51·6 p.c.)	153 (22·9 p.c.)	5 (0·7 p.c.)	—	—
5th	1905—06	276	9 (3·3 p.c.)	230 (83·3 p.c.)	26 (9·5 p.c.)	10 (3·6 p.c.)	1 (0·3 p.c.)	—
6th	1906—07	604	59 (9·8 p.c.)	201 (33·3 p.c.)	290 (48 p.c.)	31 (5·1 p.c.)	22 (3·6 p.c.)	1 (0·2 p.c.)

TABLE XI.

Data referring to villages in Rohtak District infected in the beginning of the 1903—04 epidemic.

No. of village	Tehsil	Population	Month when first infected	Remarks
112	Rohtak	9723	November '03	—
28	„	7824	„	—
32	„	4191	„	—
113	„	609	January '04	Probably infected from 112 in immediate neighbourhood.
114	„	519	„	
194	Jhaggar	2469	„	—
171	„	3896	February '04	—
135	„	3602	„	—
62	„	12,227	„	—
81	Sampla	598	„	Infected probably from Beri neighbourhood.

N.B. As this was the first epidemic none of these villages had been previously infected.

TABLE XII.

Data referring to villages in Rohtak District infected at the beginning of the 1904—05 epidemic.

No. of village	Tehsil	Population	Month when first infected in 1904-05 epidemic	Month when last death was reported in 1903-04 epidemic	No. of months free from deaths	Remarks
81	Sampla	598	August	May	2	Infected Feb.—May 04.
164	Jhaggar	531	„	Nil	—	—
62	„	12,227	September	May	3	Infected Feb.—May 04.
93	Sampla	5316	„	June	2	Infected Mar.—June 04.
74	„	5060	„	June	2	Infected Mar.—June 04.
26	Gohana	2343	„	Nil	—	—
85	Rohtak	20,024	October	May	4	Had a few deaths Feb.—May 04.
112	„	9723	„	June	3	Infected Feb.—May 04.
32	Jhaggar	1104	„	May	4	Had a few deaths Ap.—May 04.
76	Sampla	286	„	Nil	—	—
53	Rohtak	5024	November	May	5	Few deaths Ap.—May 04.
40	„	1931	„	Nil	—	—
90	„	1173	„	Nil	—	—
135	Jhaggar	3602	„	April	6	Few deaths Feb.—Ap. 04.
102	„	885	„	Nil	—	—
94	Rohtak	1735	December	Nil	—	—
74	Jhaggar	1823	„	Nil	—	—
20	Sampla	3309	„	Nil	—	—
16	Gohana	7509	„	Nil	—	—
56	„	485	„	Nil	—	—
59 A	„	383	„	Nil	—	—

TABLE XIII.

Data referring to villages in Rohtak District infected at the beginning of the 1905—06 epidemic.

No. village	Tehsil	Population	Month when first infected in 1905-06 epidemic	Month when last death was reported	No. of months free from deaths	Remarks
31	Gohana	4241	October	June 05	3	—
104	Sampla	266	„	May 05	4	Only village infected in Sampla Tehsil in this epidemic.
3	Gohana	2443	December	June 05	5	—
13	„	3185	January	June 05	6	—
21	Rohtak	2783	„	May 05	7	—
112	„	9723	„	May 05	7	—

TABLE XIV.

Data referring to villages in Rohtak District infected at beginning of the 1906—07 epidemic.

No. of village	Tehsil	Population	Month when first infected in 1906-07 epidemic	Month when last death was reported	No. of months free from deaths
11	Rohtak	5126	August	May 06	2
17	Gohana	5836	September	May 05	15
31	„	4241	„	June 06	2
12	Sampla	3765	„	May 05	15
32	„	2606	October	June 05	15
39	Gohana	5657	„	June 06	3
58	„	1660	„	May 05	16
47	„	2470	November	May 06	5
26	„	2343	„	Jan. 05	21
27	„	3247	December	May 05	18
57	„	1178	„	May 05	18

TABLE XV.

Data referring to villages in Rohtak District in which the infection was probably carried over each year from one epidemic to the other as acute rat plague.

No. of village	Tehsil	Population	Months when first and last deaths took place in 1st epidemic	No. of deaths in 1st epidemic	Month when 1st death took place in 2nd epidemic	No. of months free from deaths
Between 1st and 2nd epidemics :						
81	Sampla	698	Feb.—May 04	61	August 04	2
93	„	5316	Mar.—June 04	100	Sept. 04	2
74	„	5060	Ap.—June 04	75	Sept. 04	2
62	Jhaggar	12,227	Feb.—May 04	42	Sept. 04	3
112	Rohtak	9723	Feb.—June 04	519	Oct. 04	3
Between 2nd and 3rd epidemics :						
31	Gohana	4241	May—June 05	31	Oct. 05	3
104	Sampla	266	Ap.—May 05	5	Oct. 05	4
Between 3rd and 4th epidemics :						
11	Rohtak	5126	Ap.—May 06	13	Aug. 06	2
31	Gohana	4241	May—June 06	55	Sept. 06	2
39	„	5657	Ap.—June 06	32	Oct. 06	3

TABLE XVI.

Data referring to villages in Mozuffarnagar District infected at the beginning of the epidemic of 1903—04.

Name	Population	Thana	Month when infection began	Month when last previous death occurred	No. of months free	Remarks
Kadargarh	618	Thana Bawan	Sept. 03	Nil	—	—
Mozuffarnagar	23,444	Mozuffarnagar	Oct. 03	June 03	3	—
Khatauli	8695	Khatauli	"	"	3	—
Sandholi	418	"	Nov. 03	Nil	—	—
Mukimpur	2668	Meranpur	"	Nil	—	—
Kailaoda Kalan	2140	Khatauli	"	May 03	5	Only 1 case in ep. of 1902—03.
Naola	3752	"	Dec. 03	Nil	—	
Titawa	1192	"	"	Nil	—	—
Jauli	2579	Bhopa	"	Nil	—	—
Khandla	11,563	Khandla	"	Nil	—	—
Sarai	2752	Khatauli	Jan. 04	Nil	—	—
Barsu	1457	"	"	Nil	—	—
Pipalhera	1184	"	"	Nil	—	—
Nagli	542	"	"	Nil	—	—
Jansath	6507	Jansath	"	Nil	—	—
Kukra	3205	Shahpur	"	Nil	—	—

TABLE XVII.

Data referring to villages in Mozuffarnagar District infected at the beginning of the epidemic of 1904—05.

Name	Population	Thana	Month when infection began	Month when last previous death occurred	No. of months free	Remarks
Tijalhera	2385	Purkazi	July 04	June 04	0	—
Belra	1809	Bhopa	"	"	0	—
Gianna Mazra	790	Charthawal	"	Nil	—	—
Arnaki	167	"	"	Nil	—	—
Meranpur	7209	Meranpur	Aug. 04	June 04	1	Only 1 death in Aug. 04.
Jandheri	1019	Jansath	"	Nil	—	
Goela	3098	Shahpur	"	Nil	—	—
Harsauli	3069	Titawi	"	Nil	—	—
Rani	1753	Charthawal	"	Nil	—	—
Pur	6384	Purkazi	Sept. 04	May 04	3	Only 2 deaths in Sept. 04.
Tisa	3384	Bhopa	"	June 04	2	
Chaurawala	1543	"	"	Nil	—	—
Kethora	2668	Meranpur	"	April 04	4	Only 2 deaths in April 04.
Chandam	471	Jansath	"	Nil	—	
Kalyanpur	830	Shahpur	"	Nil	—	—
Bitanda	2663	Budhana	"	April 03	17	—
Bhukarheri	6316	Bhopa	Oct. 04	Nil	—	—
Murahlpur	3	"	"	Nil	—	—
Barkara	1220	"	"	Nil	—	—
Karehra	1220	"	"	Nil	—	—
Kanarhen	527	Charthawal	"	Nil	—	—
Rasulpur Khurd	418	Meranpur	"	April 04	5	Only 1 death in April 04. Ditto.
Antwara	1721	Jansath	"	"	5	
Basayach	889	"	"	Nil	—	—
Karthal	1823	Budhana	"	May 03	17	—
Mandoli	313	"	"	April 03	18	—

TABLE XVIII.

Data referring to villages in Mozuffarnagar District infected at the beginning of the epidemic of 1905—06.

Name	Popula- tion	Thana	Month when infection began	Month when last pre- vious death occurred	No. of months free	Remarks
Jansath	6507	Jansath	Aug. 05	May 05	2	—
Loi	7095	Kandhla	Oct. 05	Nil	—	—
Kutesra	3565	Charthawal	Nov. 05	Mar. 05	7	—
Kamhera	1197	Jansath	„	„	—	Only 1 death in 1904—05.
Balla Mazra	771	Chausana	„	April 05	—	Only 2 deaths in 1904—05.
Bhuma	1893	Meranpur	„	May 05	—	Only 1 death in 1905—06.
Dathera	1245	Chausana	Dec. 05	April 05	—	Ditto.
Shamli	7478	Shamli	„	June 05	5	—
Karnali	1078	„	„	Nil	—	—
Mustgarh	359	Thanabhawan	Jan. 06	Nil	—	—
Harsauli	3069	Titawi	„	May 05	—	Only 1 death in 1904—05.
Balwa	2503	Shamli	Feb. 06	Mar. 05	—	Ditto.
Lank	3863	„	„	June 04	19	—
Mandwara	607	Budhana	„	Nil	—	—
Nirmanani	877	Titawi	„	Nil	—	—
Ghesu Khera	571	Charthawal	„	Jan. 05	—	Only 1 death in 1905—06.
Pura	805	Khatauli	„	Nil	—	—
Gadla	1767	Bhopa	„	Jan. 05	12	—
Bhu Karheri	6316	„	„	Feb. 05	11	—

TABLE XIX.

Data referring to villages in Mozuffarnagar District infected at the beginning of the epidemic of 1906—07.

Name	Population	Thana	Month when infection began	Month when last previous death occurred	No. of months free	Remarks
Kandla	11,563	Kandla	July 06	May 06	1	1 death in July. 1 death in Aug. 7 deaths in Nov. Only 1 death in 1906—07.
Kasauli	1323	Charthawal	Sept. 06	„	—	—
Rasulpur	851	Shahpur	Oct. 06	April 04	29	—
Nizampur	299	Khatauli	„	Nil	29	—
Meranpur	7209	Meranpur	„	April 04	17	—
Wazirabad	669	Bhopa	„	Feb. 05	19	—
Chachrauli	871	„	„	Nov. 04	22	—
Yusafpur	824	„	„	Nil	22	—
Chaurawala	1543	„	„	May 06	4	—
Alayarpur	552	Shahpur	Nov. 06	Nil	4	—
Baghrli	4935	Titawi	„	May 05	17	—
M. Nagar	23,444	M. Nagar	„	June 06	4	—
Chandpur	1109	„	„	May 06	5	—
Bilaspur	1390	„	„	Feb. 05	20	—
Khojahera	999	Jansath	„	May 05	17	—
Sikri	3026	Bhopa	„	May 06	5	—
Jauli	2579	„	„	June 06	4	—
Pur	6384	Purkazi	„	May 05	17	—
Garhi-Hasanpur	1461	Chausana	„	May 05	17	—
Toda	800	„	Dec. 06	Nil	—	—
Bhikki-Mazra	487	Shamli	„	May 05	17	—
Amernagar	1939	Titawi	„	Mar. 05	20	—
Kanami	2508	„	„	May 05	18	—
Makhyali	1851	M. Nagar	„	Feb. 05	21	—
Dhudhera	872	„	„	Nil	—	—
Khatauli	3695	Khatauli	„	Mar. 05	20	—
Talra	1214	Jansath	„	May 06	—	Only 1 death, May 06.
Palri	520	„	„	May 05	18	—
Mahalki	1365	„	„	April 05	19	—
Antnara	1721	„	„	„	19	—
Jansath	6507	„	„	June 06	5	—
Chitaura	1762	„	„	May 06	6	—
Karandah	1349	„	„	Nil	—	—
Kethora	2668	Meranpur	„	Mar. 05	18	—
Gadhi-Rasulpur	418	„	„	Nil	—	—
Mukimpur	2668	„	„	Feb. 05	21	—
Kakranli	3985	Bhopa	„	„	21	—
Teora	2699	„	„	June 06	—	Only 1 death, May 05—06.
Berah-Sadat	1522	„	„	Feb. 05	21	—
Bhoapur	676	„	„	Nil	—	—
Malpura	635	„	„	Nil	—	—
Khudda	2441	Purkazi	„	May 04	30	—
Harainti	519	„	„	Nil	—	—
Lakhnanti	300	„	„	Nil	—	—
Aterna	1304	Budhana	„	Nil	—	—

TABLE XX.

Data referring to villages in Mozuffarnagar District in which the infection was probably carried over from one epidemic to another as acute rat plague.

Name	Thana	Popula- tion	Months when first and last deaths took place in 1st epidemic	No. of deaths in 1st epidemic	Month when first death took place in 2nd epidemic	No. of months free from deaths
Mozuffarnagar	M. Nagar	23,444	Feb.—June 03	25	Oct. 03	3
Khatauli	Khatauli	8695	Mar.—June 03	4	Oct. 03	3
Tijalhera	Purkazi	2385	April—July 04	120	Aug. 04	0
Belra	Bhopa	1809	May—July 04	68	Aug. 04	0
Meranpur	Meranpur	7209	Mar.—June 04	151	Aug. 04	1
Pur	Purkazi	6384	Mar.—May 04	279	Sept. 04	3
Tisa	Bhopa	3384	April—June 04	29	Sept. 04	2
Jansath	Jansath	6507	Jan.—May 05	146	Aug. 05	2
Kandla	Kandla	11,563	April—May 06	52	July 06	1

TABLE XXI.

Data referring to villages in Amritsar District infected at beginning of 1902—03 epidemic.

No.	Tehsil	Popula- tion	Month when became infected	Month of last pre- vious death	No. of months free	Remarks
113	Amritsar	162,429	Aug. 02	July 02	0	Amritsar City
24	"	1525	Oct. 02	June 02	3	3 deaths, May
17	"	6490	"	Nil	—	—June 02.
144	Ajnala	429	"	Nil	—	—
110	Amritsar	990	Nov. 02	Nil	—	—
38	"	317	"	Nil	—	—
202	"	230	"	Nil	—	—
175	"	238	"	Nil	—	—
42	"	407	"	Nil	—	—
222	"	868	"	Nil	—	—
60	"	946	"	Nil	—	—
138	"	307	"	Nil	—	—
16	"	958	"	Nil	—	—
143	"	1470	"	July 02	3	—
63	"	1700	"	"	3	1 death in
109	"	1323	"	Nil	—	Nov. 02.
39	"	1090	"	Nil	—	1 death in
						July 02.
171	"	1144	"	June 02	4	—
201	"	1966	"	Nil	—	—
239	"	1076	"	Nil	—	—
67	"	1019	"	Nil	—	—
176	"	1863	"	Nil	—	—
64	"	1117	"	Nil	—	—
65	"	1826	"	Nil	—	—
169	"	1959	"	Nil	—	—
27	"	5029	"	Nil	—	—
64	"	1130	"	Nil	—	—
46	Tarn-Tarn	1114	"	June 02	4	—
28	"	1628	"	Nil	—	—
42	"	2208	"	June 02	4	—
49	"	2440	"	July 02	3	—
80	"	1742	"	Nil	—	—
41	"	4161	"	Nil	—	—
165	"	331	"	Nil	—	—
121	"	1067	"	Nil	—	—
150	"	3206	"	Nil	—	—
100	"	930	"	Nil	—	—
33	"	518	"	Nil	—	—
120	"	4428	"	Nil	—	—
146	Ajnala	564	"	Nil	—	—
270	"	2439	"	Nil	—	—
145	"	1514	"	Nil	—	—
162	"	1166	"	Nil	—	—
180	"	405	"	Nil	—	—
161	"	954	"	Nil	—	—
162 A	"	349	"	Nil	—	—

TABLE XXII.

Data referring to villages in Amritsar District infected at the beginning of 1903—04 epidemic.

No.	Tehsil	Popula- tion	Month when became infected	Month of last pre- vious death	No. of months free	Remarks
713	Amritsar	162,429	Aug. 03	July 03	0	—
63	„	1700	Nov. 03	Nov. 02	11	1 case in Nov. 02.
258	Ajnala	2179	„	April 03	6	—
57	Amritsar	1500	Jan. 04	June 03	6	—
143	„	1470	„	Jan. 03	11	—
83	„	878	„	June 03	6	—
40	„	1868	„	May 03	7	—
196	„	1600	„	June 02	18	—
262	„	3029	„	May 03	7	—
136	„	258	„	Nil	—	—
146	„	5817	„	June 03	6	—
236	„	916	„	April 03	8	—
268	„	1158	„	May 03	7	—
216	„	2494	„	June 03	6	—
89	„	3709	„	April 03	8	—
176	„	1863	„	Feb. 03	10	—
138	Tarn-Tarn	801	„	May 03	7	—
264	„	4343	„	June 03	6	—
323	„	1846	„	Nil	—	—
41	„	4161	„	Feb. 03	10	—
67	„	1597	„	Mar. 03	9	—
120	„	4428	„	April 03	8	—
311	„	3654	„	Nil	—	—

TABLE XXIII.

Data referring to villages in Amritsar District infected at the beginning of 1904—05 epidemic.

No.	Tehsil	Popula- tion	Month when became infected	Month of last pre- vious death	No. of months free	Remarks
1	Amritsar	263	Aug. 04	June 03	13	2 deaths only in
167	,,	715	Sept. 04	Nil	—	Aug., 1 death in
25	,,	637	,,	Nil	—	Sept.
261	,,	1033	,,	May 03	15	—
189	Tarn-Tarn	730	,,	Nil	—	—
57	Amritsar	1500	Oct. 04	May 04	4	—
306	,,	524	,,	Nil	—	—
292	,,	1776	,,	Nil	—	—
103	,,	867	,,	April 04	5	—
102	,,	209	,,	Nil	—	—
317	,,	1062	,,	June 04	3	—
308	,,	843	,,	Feb. 03	19	—
291	,,	430	,,	Nil	—	—
142	Tarn-Tarn	2107	,,	June 04	3	—
241	,,	792	,,	June 03	15	—
243	,,	3538	,,	May 03	16	—
31	,,	238	,,	May 04	4	—
159	,,	1513	,,	June 04	3	—
210	Ajnala	1891	,,	July 03	14	—
146	,,	1514	,,	May 04	4	—
100	Amritsar	1517	Nov. 04	June 03	16	—
163	,,	1411	,,	June 03	16	—
327	,,	1143	,,	Nil	—	—
267	,,	1011	,,	June 03	16	—
116	,,	639	,,	Nil	—	—
78	,,	1470	,,	May 04	5	—
77	,,	611	,,	June 03	16	—
288	,,	1073	,,	June 04	4	—
276	,,	1772	,,	June 04	4	—
343	,,	1062	,,	June 04	4	—
32	,,	502	,,	Nil	—	—
176	,,	1863	,,	April 04	6	—
51	Tarn-Tarn	326	,,	June 03	16	—
190	,,	220	,,	May 03	17	—
269	,,	3400	,,	May 04	5	—
230	,,	247	,,	June 03	16	—
66	,,	1090	,,	June 04	4	—
226	,,	3291	,,	June 03	16	—
67	,,	1597	,,	May 04	5	—
215	,,	2737	,,	June 03	16	—
85	,,	1201	,,	May 04	5	2 deaths in May
273	,,	581	,,	Nil	—	04.

TABLE XXIV.

Data referring to villages in Amritsar District infected at the beginning of 1905—06 epidemic.

	Tehsil	Popula- tion	Month when became infected	Month of last pre- vious death	No. of months free	Remarks
13	Amritsar	162,429	Aug. 05	July 05	0	—
00	„	1517	Nov. 05	Mar. 05	7	—
30	„	946	„	May 04	17	—
59	„	154	„	May 04	17	1 death in May 04.
75	„	238	Jan. 06	April 05	8	—
71	„	1794	„	April 05	8	—
49	„	1306	„	May 05	7	—
59	„	533	„	Mar. 05	9	—
72	„	1520	„	April 05	8	—
86	„	1829	„	June 05	6	—
78	„	1470	„	Mar. 05	9	—
57	Tarn-Tarn	1177	„	Feb. 05	10	—
66	„	1090	„	July 05	5	—
69	„	469	„	May 04	19	—
13	„	2463	„	May 05	7	1 death in Jan. 06.
59	„	1433	„	July 05	5	—
96	Amritsar	1602	Feb. 06	June 05	6	—
75	„	2431	„	Mar. 05	9	—
01	„	432	„	May 05	7	—
61	„	1033	„	Mar. 05	9	—
11	„	1364	„	June 05	6	—
88	„	1520	„	April 05	8	—
37	„	2830	„	June 04	18	—
42	Tarn-Tarn	2208	„	June 04	18	—
87	Ajnala	763	„	June 05	6	—
09	„	692	„	April 05	8	1 death in Feb. 06.

TABLE XXV.

Data referring to villages in Amritsar District infected at the beginning of 1906—07 epidemic.

No.	Tehsil	Popula- tion	Month when became infected	Month of last pre- vious death	No. of months free	Remarks
113	Amritsar	162,429	Aug. 06	July 06	0	Last death
291	„	430	Sept. 06	Dec. 04	20	25. 8. 06, then
235	Tarn-Tarn	979	„	June 06	2	in Jan. 07.
123	„	2303	Oct. 06	June 05	15	—
183	Ajnala	843	„	May 03	40	—
165	„	323	„	Nil	—	—
43	Amritsar	1479	Nov. 06	May 05	17	—
57	„	1500	Nov. 04	Dec. 04	22	—
322	„	1335	„	Feb. 05	20	—
69	„	993	„	June 05	16	—
302	„	734	„	May 06	5	—
38	„	511	„	April 04	30	—
78	„	1470	„	April 06	6	—
72	Tarn-Tarn	1298	„	April 05	18	—
147	Ajnala	773	„	Nil	—	—
5	„	1209	„	May 04	29	1 death in 1906
181	„	943	„	May 05	17	—07.
213	„	775	„	May 03	41	—
113	„	957	„	May 05	17	—
245	„	3198	„	June 05	16	—
183 A	„	843	„	May 03	41	—
124	Amritsar	436	Dec. 06	April 05	19	—
325	„	2090	„	June 06	5	—
312	„	2110	„	May 06	6	1 death in 1906
284	„	692	„	April 05	19	—07.
96	„	1602	„	May 06	6	—
279	„	1664	„	July 06	4	—
143	„	1470	„	May 05	18	—
240	„	234	„	May 05	18	—
229	„	1779	„	June 06	5	—
318	„	897	„	May 05	18	2 deaths in Dec.
123	„	321	„	Nil	—	no more till
101	„	1346	„	June 06	5	April.
41	„	319	„	May 06	6	—
341	„	1119	„	June 06	5	—
26	„	5029	„	May 05	18	—
206	Tarn-Tarn	951	„	Feb. 05	21	—
206 A	„	1467	„	June 06	5	—
21	Ajnala	374	„	May 05	18	2 deaths only
162	„	1166	„	May 05	18	in 1906—07.
161	„	383	„	Nil	—	—
273	„	4511	„	June 06	5	—
245 A	„	3198	„	June 04	29	—
181 A	„	298	„	May 05	18	—

TABLE XXVI.

Data referring to villages in Amritsar District in which the infection was probably carried over from one epidemic to another as acute rat plague.

No.	Tehsil	Popula- tion	Months when first and last deaths took place in 1st epidemic	No. of deaths in 1st epidemic	Month when first death took place in 2nd epidemic	No. of months free from deaths
13	Amritsar	162,429	Feb.—July 02	82	Aug. 02	0
24	„	1525	May—June 02	3	Oct. 02	3
43	„	1470	April—July 02	35	Nov. 02	3
63	„	1700	July 02	2	Nov. 02	3
49	Tarn-Tarn	2440	May—July 02	14	Dec. 02	3
13	Amritsar	162,429	Aug. 02, July 03	460	Aug. 03	0
17	„	1062	May—June 04	3	Oct. 04	3
42	Tarn-Tarn	2107	April—June 04	25	Oct. 04	3
59	„	1513	April—June 04	13	Oct. 04	3
13	Amritsar	162,429	Jan.—July 05	1073	Aug. 05	0
13	„	162,429	Aug. 05, July 06	1903	Aug. 06	0
35	Tarn-Tarn	979	May—June 06	15	Sept. 06	2

TABLE XXVII.

Data referring to villages in Rohtak District infected in the epidemic of 1903—04.

No. of village	Tehsil	Population	Details of infection of 1903—04		Details of next subsequent infection		No. of months free from deaths
			Months during which deaths were returned	No. of deaths	Months during which deaths were returned	No. of deaths	
100	Rohtak	1032	Feb.—May	31	Nil	Nil	—
186	Jhaggar	210	May	1	Nil	Nil	—
71	"	384	April—May	8	Nil	Nil	—
109	"	361	Mar.—May	8	Nil	Nil	—
78	"	767	Mar.—May	54	Nil	Nil	—
14	"	419	May	1	Nil	Nil	—
95	Rohtak	1934	Mar.—May	121	Jan.—May 05	156	7
99	"	1193	Mar.—June	30	April—June 05	54	9
93	"	363	March	1	April 05	3	—
35	Sampla	2231	Mar.—May	175	Mar.—June 05	137	9
134	Jhaggar	296	June	1	Mar.—April 05	18	—
140	"	231	May	2	Jan. 05	4	—
10	"	637	Mar.—May	9	April—May 05	20	10
153	"	572	Mar.—April	20	April—May 05	16	11
75	"	645	May—June	23	April—May 05	31	9
171	"	3896	Feb.—May	108	Mar.—May 05	101	9
135	"	3602	Feb.—April	13	Nov. 04—Mar. 05	160	6
194	"	2469	Jan.—Feb.	8	April—June 05	84	13
93	"	956	March	1	April—June 05	36	—
155	"	327	Mar.—May	23	April 05	53	10
81	Sampla	598	Feb.—May	61	Aug.—Sept. 04	12	2
113	Rohtak	609	Jan.—May	26	April—May 05	38	10
107	"	4279	Feb.—June	204	April—May 05	19	9
108	"	4076	Mar.—July	296	Jan.—May 05	21	5
96	"	663	April	16	Mar.—May 05	60	10
15	"	511	Feb.—April	62	April—May 05	50	11
65	"	1285	Mar.—May	25	Mar.—April 05	39	9
32	"	4191	Nov.—May	333	Feb.—May 05	315	8
33	"	759	Feb.—May	24	April—June 05	18	10
31	"	2463	Mar.—June	180	Mar.—June 05	162	8
53	"	5024	April—May	15	Nov. 04—Mar. 05	98	5
28	"	7824	Nov.—Dec.	6	Jan.—May 05	441	12
49	"	4074	Jan.	1	Jan.—May 05	208	—
68	"	1865	May	1	Mar.—May 05	139	—
31	Sampla	2164	April	1	Jan.—June 05	199	—
74	"	5060	April—June	75	Sept. 04—June 05	334	2
12	"	3765	March	1	Jan.—Mar. 05	224	—
95	"	706	April—May	13	Feb.—April 05	29	8
68	"	1887	April	3	Jan.—May 05	117	—
83	Gohana	4013	April	1	April—May 05	31	—
161	Jhaggar	1432	March	1	Mar.—May 05	90	—
60	"	1031	April	3	Feb.—May 05	43	—
62	"	12,227	Feb.—May	42	Sept.—Dec. 04	274	—
112	Rohtak	9723	Nov.—June	525	Oct. 04—May 05	208	3
85	"	20,024	Feb.—May	12	Oct. 04—May 05	900	4
35	"	7640	Mar.—June	262	Jan.—May 05	265	6
21	"	3783	March	1	April—May 05	71	—
93	Sampla	5316	Mar.—June	100	Sept. 04—June 05	324	2
29	Gohana	1035	May—June	31	April—May 05	38	9
32	Jhaggar	1104	April—May	6	Oct. 04—June 05	59	4
128	"	603	April	1	April 07	1	—
18	"	1298	Nov.	1	Mar.—April 07	2	—
62	Sampla	1415	April	1	Feb.—May 07	91	—
72	"	766	April—May	3	May 07	15	—
98	Rohtak	1494	June	1	April—June 07	32	—
63	Jhaggar	702	Feb.	1	April—June 07	26	—
114	Rohtak	519	Jan.—Mar.	47	April—May 07	5	35

TABLE XXVIII.

Shows the period during the epidemic of 1904—05 in which 31 villages in Rohtak District infected the previous epidemic first reported plague deaths.

	1904					1905				
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
Number of villages infected in 1903—04 in which deaths were returned in 1904—05.	1	3	3	2	0	4	2	5	11	0
Total number of villages which returned deaths each month.	2	6	9	17	23	63	101	156	232	208

TABLE XXIX.

Shows the interval for which 31 villages in Rohtak District infected in the epidemics of 1903—04 and 1904—05 did not report plague deaths.

	Number of months in which no plague deaths were returned												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of villages	0	3	2	2	2	2	1	3	7	5	2	1	1

TABLE XXX.

Data referring to villages in Rohtak District infected in epidemic of 1905—06.

No. of village	Tehsil	Popula- tion	Details of infection of 1905—06		Details of infection of 1906—07		No. of months free from deaths
			Months during which deaths were returned	No. of deaths	Months during which deaths were returned	No. of deaths	
2	Gohana	2269	April—May 06	110	May—June 07	64	10
12	,,	1205	Feb.—May 06	172	May—June 07	23	11
45	,,	1291	Feb.—April 06	53	March—May 07	117	10
79	,,	1720	March—April 06	17	April—June 07	116	11
83	Rohtak	4727	April—May 06	3	Jan.—May 07	266	—
16	Gohana	7509	April—June 06	195	Feb.—June 07	609	7
33	,,	1521	March—May 06	68	March—May 07	175	9
47	,,	2470	April—May 06	39	Nov. 06—May 07	177	5
3	Rohtak	2948	April—May 06	6	April—July 07	93	10
15	Gohana	4068	Feb.—May 06	258	March—June 07	265	9
3	,,	2443	Dec. 05—April 06	68	May—June 07	26	12
31	,,	4241	May—June 06	55	Sept. 06—July 07	897	2
49	,,	4568	Feb.—May 06	337	Feb.—June 07	258	8
39	Rohtak	3838	April 06	4	Feb.—May 07	135	—
39	Gohana	5657	April—June 06	32	Oct. 06—July 07	245	3
11	Rohtak	5126	April—May 06	13	Aug. 06—June 07	234	2
48	Gohana	4115	May 06	11	Feb.—May 07	274	8
80	,,	2245	May 06	8	March—April 07	174	9
13	,,	3185	Jan.—March 06	35	April—June 07	206	10
112	Rohtak	9723	Jan.—May 06	257	March—June 07	65	9
85	,,	20,024	April—May 06	3	Feb.—June 07	463	—
93	Sampla	5316	April 06	1	Feb.—June 07	416	—
35	Rohtak	7640	Feb.—May 06	9	March—June 07	49	10
29	Gohana	1035	Jan. 06	2	April—June 07	17	—
32	Jhaggar	1104	April 06	1	April—May 07	43	—
21	Rohtak	3783	Jan.—March 06	29	March—June 07	574	11
11	Jhaggar	645	April—May 06	5	Nil	—	—
94	Rohtak	1735	April—May 06	38	Nil	—	—
106	,,	1214	May 06	4	Nil	—	—
104	Sampla	266	Oct. 05—Feb. 06	82	Nil	—	—

TABLE XXXI.

Shows the period during the epidemic of 1906—07 in which 20 villages in Rohtak District infected the previous epidemic first reported plague deaths.

	1906					1907				
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
Number of villages infected in 1905—06 in which deaths were returned in 1906—07.	1	1	1	1	0	0	3	7	3	3
Total number of villages which returned deaths each month.	1	4	7	9	11	21	49	132	198	199

TABLE XXXII.

Shows the interval for which 20 villages in Rohtak District infected in the epidemics of 1905—06 and 1906—07 did not report plague deaths.

	Number of months in which no plague deaths were returned												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of villages	0	2	1	0	1	0	1	2	4	5	3	1	0

TABLE XXXIII.

Data referring to villages in Mozuffarnagar District infected in 1902—03 epidemic as regards subsequent infection.

	Popula- tion	Thana	Details of infection of 1902—03		Details of next infection		No. of months free
			Dates of first and last deaths	No. of deaths	Dates of first and last deaths	No. of deaths	
an	97	Purkazi	30. 4. 03— 1. 5. 03	3	Nil	—	—
c	882	Thana- Bhavan	22. 3. 03—28. 3. 03	3	Nil	—	—
	441	Budhana	5. 4. 03— 5. 4. 03	1	Nil	—	—
ali	644	,,	4. 11. 02—28. 4. 03	59	3. 10. 04—14. 10. 03	10	17
	1445	,,	10. 2. 03—10. 2. 03	1	5. 11. 04—15. 11. 04	15	20
i	561	,,	29. 11. 02—29. 11. 02	1	1. 5. 07— 7. 5. 07	15	53
hangaim	779	Jansath	10. 5. 03—10. 5. 03	1	12. 5. 07—27. 5. 07	19	47
	681	Khatauli	14. 4. 03— 8. 5. 03	70	6. 2. 04—10. 2. 04	9	8
Samand	1163	,,	21. 4. 03—27. 4. 03	1	29. 3. 04— 7. 4. 04	27	10
-Kalan	1742	Titavi	12. 3. 03—12. 3. 03	1	30. 2. 05—13. 5. 05	32	23
a	4497	Purkazi	16. 4. 03— 8. 6. 03	69	12. 3. 05—31. 5. 05	172	20
garh	618	Thana- Bhavan	16. 9. 03—16. 9. 03	1	2. 1. 05—18. 3. 05	15	15
adpur	1113	Khatauli	10. 4. 03— 2. 5. 03	16	14. 1. 05—15. 2. 05	57	19
na	6664	Budhana	7. 3. 03— 7. 3. 03	1	21. 5. 05— 4. 6. 05	199	25
a	2663	,,	22. 11. 02—26. 4. 03	279	1. 9. 04— 3. 11. 04	30	16
heri	694	,,	29. 11. 02—29. 11. 02	2	9. 5. 05—13. 5. 05	16	29
l	1823	,,	7. 4. 03—12. 5. 03	46	1. 10. 04—12. 10. 04	7	16
	2754	,,	4. 11. 02— 4. 11. 02	1	25. 4. 05—11. 6. 05	203	28
ali	8695	Khatauli	13. 3. 03—10. 6. 03	11	23. 10. 03— 6. 4. 04	305	3
c	855	,,	12. 4. 03—12. 4. 03	1	8. 2. 04—29. 2. 04	37	9
a-Kalan	2140	,,	22. 4. 03—30. 5. 03	2	14. 11. 03—20. 11. 03	4	5
our	7209	Meranpur	26. 2. 03—26. 5. 03	150	31. 3. 04— 8. 6. 04	151	9
Sarai	1443	,,	18. 4. 03—23. 5. 03	23	18. 4. 04—21. 4. 04	2	10
ur	2294	Shahpur	10. 4. 03—29. 5. 03	21	11. 3. 04— 8. 5. 04	46	9
gar	23,444	M. Nagar	21. 2. 03—11. 6. 03	25	25. 10. 03—23. 4. 04	331	3

TABLE XXXIV.

Showing the interval during which 11 villages in Mozuffarnagar District infected in 1902—03 and again in 1903—04 or subsequent epidemic did not report plague deaths.

	Number of months during which no plague deaths were returned											
	1	2	3	4	5	6	7	8	9	10	11	12 & over
Number of villages	0	0	2	0	0	0	0	1	2	1	0	5

TABLE XXXV.

Showing the period during the epidemic of 1903—04 in which 6 villages in Mozuffarnagar District infected the previous epidemic first reported plague deaths.

	1903					1904				
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
Number of villages infected in 1902—03.	0	0	2	0	0	0	1	2	1	0
Total number of villages which returned deaths.	0	0	2	5	7	11	30	51	91	52

TABLE XXXVI.

*Data referring to villages in Mozuffarnagar District infected in 1905—06
as regards subsequent infection.*

Village	Population	Thana	Details of infection in 1905—06		Details of next infection		No. of months free
			Dates of first and last deaths	No. of deaths	Dates of first and last deaths	No. of deaths	
Nagar	23,444	M. Nagar	8. 3. 06—11. 6. 06	112	5. 11. 06—23. 5. 07	480	4
Chaheri	915	„	21. 3. 06—27. 4. 06	9	21. 3. 07—19. 4. 07	36	10
Madpur	1109	„	4. 4. 06—20. 5. 06	12	30. 11. 06— 3. 2. 07	41	5
Chahra	2853	Titawi	22. 4. 06—24. 4. 06	3	26. 3. 07—18. 6. 07	135	10
Chaurah	308	„	22. 4. 06—22. 4. 06	1	8. 2. 07— 6. 3. 07	26	9
Chauli	3069	„	21. 1. 06—23. 4. 06	208	25. 3. 07—23. 5. 07	348	10
Chahna Kurd	1046	„	16. 3. 06—13. 4. 06	32	8. 3. 07— 5. 4. 07	53	10
Chahani	1315	„	11. 2. 06—11. 2. 06	1	5. 4. 07—24. 5. 07	141	13
Chahra Khara	571	Charthawal	4. 2. 06— 4. 2. 06	1	Nil	—	—
Chahra	3565	„	5. 11. 05—23. 1. 06	140	8. 3. 07— 5. 6. 07	273	13
Chahand	557	„	3. 5. 06— 3. 5. 06	1	5. 5. 07—12. 5. 07	12	11
Chahuli	1323	„	9. 4. 06—11. 5. 06	36	20. 9. 06—20. 9. 06	1	3
Chahra	2579	Bhopa	27. 3. 06— 5. 6. 06	53	10. 11. 06—21. 5. 07	103	4
Chahra	1809	„	22. 3. 06— 6. 6. 06	100	24. 1. 07—17. 5. 07	128	6
Chahra	2699	„	10. 6. 06—10. 6. 06	1	1. 12. 06— 1. 12. 06	1	5
Chahareri	6316	„	8. 2. 06—13. 5. 06	356	20. 2. 07—23. 5. 07	295	8
Chahra	3026	„	6. 5. 06—23. 5. 06	24	7. 11. 06—24. 4. 07	47	5
Chahra	1767	„	3. 2. 06— 9. 5. 06	105	4. 3. 07—15. 5. 07	117	9
Chahrawala	1543	„	15. 5. 06—23. 5. 06	4	21. 10. 06— 5. 11. 06	16	4
Chahabas	282	„	4. 3. 06— 4. 3. 06	1	Nil	—	—
Chahuli	7478	Shamli	24. 12. 05—17. 5. 06	96	5. 2. 07— 7. 6. 07	239	8
Chahali	1078	„	10. 12. 05— 5. 4. 06	59	17. 1. 07—28. 3. 07	86	8
Chahra	3863	„	10. 2. 06— 9. 5. 06	190	28. 2. 07— 3. 6. 07	206	8
Chahra	2503	„	10. 2. 06—28. 5. 06	180	3. 4. 07— 3. 5. 07	162	10
Chahra	2563	„	12. 4. 06—12. 4. 06	4	22. 3. 07— 3. 6. 07	218	10
Chahnan	1765	„	17. 3. 06—17. 3. 06	1	Nil	—	—
Chahandah	1471	„	5. 4. 06— 5. 4. 06	1	27. 4. 06— 7. 6. 07	128	11
Chahansa	1088	„	22. 3. 06—22. 3. 06	4	30. 5. 07—30. 5. 07	1	13
Chahra	738	„	5. 5. 06—25. 5. 06	30	23. 5. 07— 5. 6. 07	38	11
Chahri	2212	„	2. 4. 06— 2. 4. 06	5	15. 5. 07— 3. 6. 07	38	12
Chahri	2438	„	14. 3. 06— 5. 6. 06	98	3. 3. 07— 2. 6. 07	224	8
Chahnwara	1581	Thana-Bhavan	15. 3. 06—25. 4. 06	47	28. 3. 07—13. 5. 07	123	10
Chahgarh	359	„	21. 1. 06—23. 1. 06	6	13. 4. 07— 6. 5. 07	50	14
Chahabhawan	8861	„	24. 4. 06—12. 5. 06	13	13. 2. 07— 7. 6. 07	719	8
Chahra	356	Jhinjhana	1. 4. 06—28. 4. 06	13	28. 5. 07— 3. 6. 07	12	12
Chah Mazra	771	Chausana	10. 11. 05—13. 12. 05	11	27. 3. 07— 8. 5. 07	134	14
Chahra	1245	„	13. 12. 05—13. 12. 05	1	7. 4. 07— 8. 6. 07	32	15
Chahana	19,304	Kairana	19. 4. 06—25. 4. 06	2	14. 2. 07—13. 6. 07	2136	9
Chahvan	1158	„	28. 4. 06—28. 5. 06	32	7. 5. 07—21. 6. 07	67	11

TABLE XXXVI (*continued*).

Name	Popula- tion	Thana	Details of infection in 1905-06		Details of next infection		No. of month free
			Dates of first and last deaths	No. of deaths	Dates of first and last deaths	No. of deaths	
Bharu	2694	Kairana	28. 5. 06—28. 5. 06	2	13. 4. 07— 3. 6. 07	189	10
Jansath	6507	Jansath	13. 8. 05—11. 6. 06	90	1. 12. 06— 8. 5. 07	268	5
Talra	1214	,,	19. 4. 06— 1. 5. 06	2	21. 12. 06— 3. 5. 07	169	6
Kawal	4268	,,	17. 3. 06—29. 5. 06	117	25. 1. 07— 5. 5. 07	295	7
Chitaura	1762	,,	24. 4. 06—15. 5. 06	23	23. 12. 06— 4. 6. 07	101	6
Kheri Ferozabad	983	,,	28. 3. 06—20. 5. 06	31	25. 4. 07— 8. 6. 07	145	10
Nagla Mubarik	538	,,	17. 4. 06—19. 5. 06	19	28. 4. 07— 3. 5. 07	21	10
Kamehra	1197	,,	3. 11. 05—15. 1. 06	53	14. 2. 07—13. 5. 07	171	12
Khilwara	416	,,	20. 3. 06— 5. 5. 06	41	28. 2. 07— 8. 3. 07	20	8
Bera Asa	1349	,,	14. 4. 06—23. 4. 06	2	23. 4. 07— 3. 6. 07	48	11
Baupara	1341	Khatauli	6. 4. 06—20. 4. 06	31	7. 4. 07— 3. 6. 07	166	11
Pur Bahani	4489	,,	2. 3. 06—16. 5. 06	128	3. 4. 07—30. 5. 07	247	10
Pura	805	,,	2. 2. 06—31. 5. 06	127	15. 1. 07— 7. 6. 07	80	7
Sandhera	1881	Meranpur	9. 4. 06— 6. 5. 06	59	2. 3. 07— 3. 4. 07	85	9
Tiraula	1286	,,	17. 5. 06—28. 5. 06	18	4. 3. 07— 7. 4. 07	58	9
Bhuma	1892	,,	17. 11. 05—17. 11. 05	1	27. 1. 07— 3. 6. 07	204	13
Mandwara	607	Budhana	4. 2. 06—14. 3. 06	54	1. 3. 07— 7. 5. 07	146	11
Warli	804	,,	23. 3. 06—21. 4. 06	27	28. 5. 07—28. 5. 07	2	12
Bahsana	201	,,	28. 3. 06—26. 4. 06	52	23. 4. 07— 3. 6. 07	24	11
Habibpur	825	,,	30. 3. 06— 8. 4. 06	24	22. 4. 07—12. 5. 07	47	11
Sarai	1148	,,	30. 3. 06—12. 5. 06	40	19. 4. 07— 3. 6. 07	49	10
Tanda Mazra	1086	,,	5. 4. 06—14. 5. 06	38	8. 4. 07— 3. 6. 07	48	10
Kandhla	11,563	Kandhla	14. 4. 06—25. 5. 06	52	20. 7. 06—14. 11. 06	9	1
Phagana	3236	,,	11. 5. 06—11. 5. 06	2	6. 6. 07— 6. 6. 07	2	12
Rampur Kheri	698	,,	13. 4. 06—13. 4. 06	6	Nil	—	—
Gujarpur	328	,,	21. 4. 06—21. 4. 06	3	Nil	—	—
Ailani	3796	,,	24. 4. 06—27. 4. 06	8	Nil	—	—
Khandrauli	2815	,,	11. 5. 06—11. 5. 06	4	15. 5. 07—17. 6. 07	36	11
Garhi Ram	1957	,,	19. 3. 06— 5. 4. 06	20	Nil	—	—
Loi	1905	,,	30. 10. 05—11. 12. 05	22	16. 3. 07— 7. 6. 07	114	14

TABLE XXXVII.

Showing the interval in months during which 53 villages in Mozuffarnagar District infected in 1905—06 and again in 1906—07 did not report plague deaths.

Number of months	0	1	2	3	4	5	6	7	8	9	10	11	12 & over
Number of villages	0	1	0	0	3	3	3	2	7	4	13	8	9

TABLE XXXVIII.

Showing the period during the epidemic of 1906—07 in which 53 villages in Mozuffarnagar District infected in the previous epidemic first returned plague deaths.

	1906						1907					
	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June
Number of villages infected in 1905—06.	1	0	0	1	4	3	5	7	13	12	6	1
Total number of villages which returned deaths.	1	1	1	7	16	35	77	159	288	438	427	169

TABLE XXXIX.

Data of future plague infection of villages in Rohtak District infected at the end of each epidemic.

No. of village	Tehsil	Popula- tion	No. of deaths during epidemic	Month when last death took place	Date when next infected	No. of months free from deaths
A. End of epidemic of 1903—04.						
108	Rohtak	4076	296	July 04	Jan. 05	5
35	„	7640	262	June 04	Jan. 05	6
31	„	2463	180	„	Mar. 05	8
99	„	1193	30	„	April 05	9
107	„	4279	204	„	April 05	9
112	„	9723	519	„	Oct. 04	3
29	Gohana	1035	31	„	April 05	9
93	Sampla	5316	100	„	Sept. 04	2
74	„	5060	75	„	Sept. 04	2
75	Jhaggar	645	23	„	April 05	9
B. End of epidemic of 1904—05.						
79	Rohtak	1686	16	June 05	Mar. 07	20
33	„	759	18	„	Mar. 07	20
31	„	2463	162	„	April 07	21
23	„	413	15	„	Nil	—
39	„	3838	154	„	April 06	9
3	„	2948	83	„	April 06	9
35	„	200	20	„	Nil	—

TABLE XXXIX (*continued*).

No. of village	Tehsil	Population	No. of deaths during epidemic	Month when last death took place	Date when next infected	No. of months free from deaths
B. End of epidemic of 1904—05 (<i>continued</i>).						
30	Gohana	3327	231	,,	Mar. 07	20
82	,,	1928	112	,,	April 07	21
33	,,	1521	110	,,	Mar. 06	8
47	,,	2470	100	,,	April 06	9
21	,,	1296	76	,,	Feb. 07	19
15	,,	4068	20	,,	Feb. 06	7
3	,,	2443	23	,,	Jan. 06	6
31	,,	4241	31	,,	April 06	9
6	,,	3310	452	,,	Feb. 07	19
28	,,	330	28	,,	Feb. 07	19
74	,,	1923	15	,,	Mar. 07	20
78	,,	930	106	,,	June 07	23
66	,,	1467	28	,,	April 07	21
36	,,	2735	148	,,	Mar. 07	20
32	,,	1113	26	,,	April 07	21
11	,,	1191	78	,,	Nil	—
65	,,	1623	19	,,	April 07	21
67	,,	3133	187	,,	Mar. 07	20
64	,,	2034	211	,,	April 07	21
4	,,	2415	121	,,	April 07	21
48	,,	4115	332	,,	Mar. 06	8
13	Gohana	3185	61	June 05	Jan. 06	6
19	Sampla	1431	176	July	April 07	20
33	Jhaggar	4006	39	June	May 07	22
181	,,	974	72	,,	Nil	—
19	,,	927	2	,,	Nil	—
144	,,	1181	61	,,	Nil	—
32	,,	1104	47	,,	April 07	21
194	,,	2469	84	,,	Nil	—
93	,,	956	36	,,	Nil	—
C. End of epidemic of 1905—06.						
83	Rohtak	4727	3	May	Jan. 07	7
35	,,	7640	9	,,	Mar. 07	9
94	,,	1735	38	,,	Nil	—
11	,,	5126	13	,,	Jan. 07	7
106	,,	1214	4	,,	Nil	—
3	,,	2948	6	,,	April 07	10
112	,,	9723	257	,,	Mar. 07	9
16	Gohana	7509	195	June	Feb. 07	7
2	,,	2269	110	,,	May 07	10
31	,,	4241	55	,,	Sept. 06	2
39	,,	5657	32	,,	Oct. 06	3
11	Jhaggar	645	5	May	Nil	—

TABLE XL.

Data referring to villages in Mozuffurnagar District infected at the end of each epidemic.

Name	Population	Thana	No. of deaths during epidemic	Month in which last death occurred	Date when next infected, month of first death	No. of months free
I. 1902—1903.						
Mozuffarnagar	23,444	M. Nagar	25	June 03	Oct. 03	3
Basera	4497	Purkazi	69	„	Mar. 05	20
Khatauli	8695	Khatauli	4	„	Oct. 03	3
Amarpur	2294	Shahpur	21	May 03	Mar. 04	9
Chandan	97	Purkazi	3	„	Nil	—
Tigain	681	Khatauli	70	„	Feb. 04	8
Majahadpur	1113	„	16	„	Jan. 05	19
Meranpur	7209	Meranpur	150	„	Mar. 04	9
Kheri-Sarai	1443	„	23	„	April 04	10
Kailavda Kalan	2140	Khatauli	1	„	Nov. 03	5
Karthal	1823	Budhana	46	„	Oct. 04	17
II. 1903—1904.						
Tijalhera	2385	Purkazi	120	June 04	July 04	0
Gadla	1767	Bhopa	2	„	Jan. 05	6
Belra	1809	„	68	„	July 04	0
Berahthra	1061	„	25	„	May 07	34
Tisa	3384	„	29	„	Sept. 04	2
Tandhera	1881	Meranpur	72	„	Mar. 05	8
Sambalhera	2329	„	93	„	Nov. 04	4
Meranpur	7209	„	151	„	Aug. 04	1
Nayagind	1020	„	2	„	Mar. 07	32
Katka	830	Jansath	5	„	Jan. 05	6
Kambara	1197	„	40	„	Nov. 05	16
Mubarik	538	„	26	„	April 06	21
Kawal	4268	„	209	„	Nov. 04	4
Pal	477	„	4	„	Feb. 07	31
Mahalki	1365	„	40	„	Mar. 05	8
Ahrora	484	„	38	„	Mar. 05	8
Gangdheri	1085	Khatauli	10	„	Jan. 07	30
Kilaoda Kalan	2140	„	2	„	Dec. 04	5
Sudhan	510	„	5	„	Mar. 05	8
Lank	3863	Shamli	263	„	Feb. 06	19
Balva	2503	„	63	„	Nov. 04	4
Purbalin	4489	Khatauli	67	„	Dec. 04	5
III. 1904—1905.						
Rahkra	1425	Bhopa	34	June 05	Feb. 07	19
Badharwala	343	M. Nagar	1	„	May 07	22
Medpur	221	„	1	„	Nil	—

TABLE XL (*continued*).

Name	Population	Thana	No. of deaths during epidemic	Month in which last death occurred	Date when next infected, month of first death	No. of months free
III. 1904—1905 (<i>continued</i>).						
Sherpur	1156	M. Nagar	2	June 05	Mar. 07	20
Nirana	680	„	20	„	Mar. 07	20
Jaranda	1744	„	26	„	April 07	21
Berahasa	1349	Jansath	3	„	April 06	9
Sekheri	1207	„	5	„	Feb. 07	19
Mahmmadpur	600	Khatauli	5	„	April 07	21
Satheri	1828	„	33	„	Feb. 07	19
Sohangni	1827	„	100	„	April 07	21
Karandah	1532	Titawi	32	„	Nil	—
Jafarpur	756	Thanabhawan	26	„	Feb. 07	19
Jalabad	6822	„	319	„	Jan. 07	18
Nogal	276	„	7	„	April 07	21
Chirdeka	509	„	23	„	April 07	21
Babri	2438	Shamli	276	„	Mar. 06	8
Banat	3590	„	256	„	Mar. 07	20
Titauli	1180	„	156	„	Mar. 07	20
Shamli	7478	„	149	„	Dec. 05	5
Kaserwa Khurd	914	„	47	„	May 07	22
Phagana	3236	Kandhla	30	„	May 06	10
Gangani	6401	„	157	„	Mar. 07	20
Parasauli	2198	„	114	„	Nil	—
Kharar	3385	Budhana	143	„	April 07	21
Bari	1199	„	25	„	Nil	—
Raipur	552	„	54	„	Nil	—
Jaula	4691	„	163	„	May 07	22
Budhana	6664	„	199	„	Jan. 07	18
Baranda	2754	„	203	„	April 07	21
Karthal	1823	„	81	„	April 07	21
Atawa	1445	„	96	„	Nil	—
Nagwa	1859	„	114	„	Nil	—
Kairana	19,304	Kairana	94	„	April 06	9
Un	4502	Jhanjnana	272	„	April 07	21

IV. 1905—1906.

Belra	1809	Bhopa	100	June 06	Jan. 07	6
Jauli	2579	„	53	„	Nov. 06	4
Teora	2699	„	1	„	Dec. 06	5
Jansath	6507	Jansath	69	„	Dec. 06	5
Mozuffarnagar	23,444	M. Nagar	112	„	Nov. 06	4
Bahori	2323	Shamli	96	„	Mar. 07	8

TABLE XLI.

*Data referring to villages in the Amritsar District infected
at the end of each epidemic.*

No.	Tehsil	No. of deaths in epidemic	Month when last death occurred	Month when next death occurred	Months remained free
Epidemic of 1901—1902.					
57	Amritsar	42	July 02	April 03	8
143	„	35	„	Jan. 03	5
63	„	2	„	Nov. 03	14
241	„	6	„	May 03	9
113	„	249	„	Aug. 02	0
49	Tarn-Tarn	14	„	Nov. 02	3
64	„	70	„	Feb. 03	6
170	Amritsar	130	June 02	Jan. 03	6
130	„	12	„	May 03	10
171	„	41	„	May 03	10
86	„	25	„	Jan. 03	6
196	„	50	„	Jan. 04	18
145	„	21	„	April 04	21
311	„	140	„	May 03	10
142	„	31	„	Jan. 07	54
77	„	53	„	Nov. 04	27
24	„	3	„	Feb. 04	19
97	Tarn-Tarn	27	„	Mar. 03	8
213	„	4	„	April 03	9
34	„	3	„	April 04	21
46	„	14	„	Nov. 02	4
42	„	2	„	Nov. 02	4
19	„	19	„	April 04	21
67	„	6	„	Jan. 03	6
100	„	7	„	April 04	21
Epidemic of 1902—1903.					
189	Amritsar	20	July 03	Nil	—
213	„	15	„	April 04	8
210	„	12	„	April 04	8
219	„	13	„	June 04	10
164	„	30	„	Dec. 04	16
266	„	40	„	Jan. 05	17
334	„	10	„	Jan. 05	17
200	„	20	„	Feb. 05	18
253	„	40	„	Jan. 05	17
27	„	40	„	Feb. 04	6
137	„	32	„	Dec. 04	16
288	„	26	„	May 04	9
342	„	25	„	Feb. 05	18
113	„	293	„	Aug. 03	0

TABLE XLI (*continued*).

No.	Tehsil	No. of deaths in epidemic	Month when last death occurred	Month when next death occurred	Months remained free
Epidemic of 1902—1903 (<i>continued</i>).					
323	Tarn-Tarn	27	July 03	Mar. 04	7
315	"	185	"	Mar. 05	19
233	"	30	"	Feb. 05	18
196	"	195	"	April 04	8
178	Ajnala	29	"	Dec. 04	16
257	"	50	"	Dec. 04	16
160	"	10	"	Jan. 05	17
56	"	25	"	April 05	20
295	"	25	"	April 05	20
281	"	100	"	April 05	20
244	"	3	"	Nil	—
210	"	70	"	Oct. 04	14
276	"	25	"	May 07	45
161	"	55	"	May 04	9
131	"	35	"	Dec. 04	16
114	"	8	"	May 04	9
244-273	"	90	"	Mar. 04	7
267	"	20	"	Nil	—
Epidemic of 1903—1904.					
232	Amritsar	111	July 04	Feb. 05	6
311	"	326	"	Dec. 04	4
5	"	88	"	Mar. 05	7
113	"	1103	"	Jan. 05	5
17	"	233	"	Jan. 05	5
112	Tarn-Tarn	135	"	Feb. 05	6
42	"	244	"	April 05	8
143	"	48	"	Nil	—
240	"	1	"	Mar. 05	7
196	"	85	"	Mar. 05	7
270	Ajnala	138	"	May 05	9
100	"	62	"	May 07	33
63	"	16	"	Mar. 07	31
91	"	29	"	April 07	32
Epidemic of 1904—1905.					
287	Amritsar	134	July 05	April 06	8
113	"	1073	"	Aug. 05	0
17	"	86	"	April 06	8
5	Tarn-Tarn	101	"	Mar. 06	7
142	"	117	"	April 07	20
267	"	88	"	June 06	10
300	"	107	"	April 07	20
241	"	24	"	Mar. 07	19

TABLE XLI (*continued*).

No.	Tehsil	No. of deaths in epidemic	Month when last death occurred	Month when next death occurred	Months remained free
Epidemic of 1904—1905 (<i>continued</i>).					
43	Tarn-Tarn	18	July 05	Mar. 07	19
64	„	256	„	May 06	9
21	„	140	„	Nil	—
86	„	95	„	Feb. 07	18
66	„	214	„	Jan. 06	5
59	„	9	„	Nil	—
81	„	77	„	Jan. 07	17
23	„	26	„	Nil	—
50	„	175	„	April 07	20
40	„	144	„	Nil	—
19	„	47	„	May 06	9
64	„	165	„	May 06	9
54	„	159	„	April 07	20
68	„	86	„	June 06	10
11	„	278	„	April 06	8
76	„	5	„	April 06	8
79	„	213	„	Mar. 07	19
26	„	35	„	Feb. 07	18
26	Ajnala	213	„	Mar. 07	19
269	„	54	„	April 07	20
295	„	58	„	Feb. 07	18
81	„	90	„	April 07	20
303	„	52	„	April 07	20
267	„	130	„	Feb. 07	18
206	„	16	„	Nil	—
262	„	87	„	April 07	20
226	„	12	„	April 07	20
309	„	86	„	April 07	20
161	„	70	„	Mar. 07	19
251	„	86	„	May 07	21
235	„	11	„	Nil	—
Epidemic of 1905—1906.					
279	Amritsar	25	July 06	Dec. 06	4
160	„	1	„	Feb. 07	6
232	„	70	„	Feb. 07	6
262	„	113	„	Nil	—
311	„	202	„	Mar. 07	7
296	„	9	„	Nil	—
5	„	39	„	Jan. 07	5
113	„	1863	„	Aug. 06	0
17	„	30	„	Jan. 07	5
225	„	113	„	April 07	8
197	Ajnala	11	„	Jan. 07	5

TABLE XLII.

Showing the interval of freedom from plague deaths enjoyed by villages in Rohtak District which were still infected at the end of the different epidemics.

		Number of months during which no plague deaths were returned												
		0	1	2	3	4	5	6	7	8	9	10	11	12 & over
1903—04.														
Number of villages		0	0	2	1	0	1	1	0	1	4	—	—	—
1904—05.														
Number of villages		0	0	0	0	0	0	2	1	2	4	0	0	20
1905—06.														
Number of villages		0	0	1	1	0	0	0	3	0	2	2	—	—

TABLE XLIII.

Showing the interval of freedom from plague deaths enjoyed by villages in the Mozuffarnagar District which were still infected at the end of the different epidemics.

		Number of months during which no plague deaths were returned												
		0	1	2	3	4	5	6	7	8	9	10	11	12 & over
1902—03.														
Number of villages		0	0	0	2	0	1	0	0	1	2	1	0	3
1903—04.														
Number of villages		2	1	1	0	3	1	2	0	4	0	0	0	8
1904—05.														
Number of villages		0	0	0	0	0	1	0	0	1	2	1	0	23
1905—06.														
Number of villages		0	0	0	0	2	2	1	0	1	0	0	0	0

TABLE XLIV.

Showing the interval of freedom from plague deaths enjoyed by villages in the Amritsar District which were still infected at the end of the different epidemics.

	Number of months during which no plague deaths were returned												
	0	1	2	3	4	5	6	7	8	9	10	11	12 & over
1901—02.													
Number of villages	1	0	0	1	2	1	4	0	2	2	3	0	9
1902—03.													
Number of villages	1	0	0	0	0	0	1	2	3	3	1	0	18
1903—04.													
Number of villages	0	0	0	0	1	2	2	3	1	1	0	0	3
1904—05.													
Number of villages	1	0	0	0	0	1	0	1	4	3	2	0	21
1905—06.													
Number of villages	1	0	0	0	1	3	2	1	1	—	—	—	—

TABLE XLV.

Showing the total number of villages in Rohtak District and the number of villages infected in each epidemic.

Total No. of villages	Number of villages infected			
	1st epidemic 1903—04	2nd epidemic 1904—05	3rd epidemic 1905—06	4th epidemic 1906—07
499	57	285	30	249

TABLE XLVI.

Showing the actual number of villages in Rohtak District which were infected in no, one, two, three and four epidemics.

Number of villages infected in				
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics
145	138	172	37	7

TABLE XLVII.

Showing the calculated probable number of villages in Rohtak District which would have been infected in no, one, two, three and four epidemics if all villages were equally liable to infection in all four epidemics.

Number of villages infected in				
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics
89	225	159	24	1
				27—2

TABLE XLVIII.

Showing the total number of villages in Mozuffarnagar District and the number of villages infected in each epidemic.

Total No. of villages	Number of villages infected in				
	1st epidemic	2nd epidemic	3rd epidemic	4th epidemic	5th epidemic
973	25	130	313	69	579

TABLE XLIX.

Showing the actual number of villages in Mozuffarnagar District which were infected in no, one, two, three, four and five epidemics.

Number of villages infected in					
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics	Five epidemics
334	301	226	86	25	1

TABLE L.

Showing the calculated probable number of villages in Mozuffarnagar District which would have been infected in no, one, two, three, four and five epidemics if all villages were equally liable to infection in all five epidemics.

Number of villages infected in					
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics	Five epidemics
202	457	261	49	3	0.05

TABLE LI.

Showing the total number of villages in Amritsar District and the number of villages infected in each epidemic.

Total No. of villages	Number of villages infected in					
	1st epidemic	2nd epidemic	3rd epidemic	4th epidemic	5th epidemic	6th epidemic
1062	62	506	445	669	276	604

TABLE LII.

Showing the actual number of villages in Amritsar District which were infected in no, one, two, three, four, five and six epidemics.

Number of villages infected in						
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics	Five epidemics	Six epidemics
155	183	211	230	169	93	21

TABLE LIII.

Showing the calculated probable number of villages in Amritsar District which would have been infected in no, one, two, three, four, five and six epidemics if all villages were equally liable to infection in all six epidemics.

Number of villages infected in						
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics	Five epidemics	Six epidemics
48·5	195·8	329·1	295·1	148·8	40	4·5

TABLE LIV.

Showing the number of villages in Rohtak District infected each epidemic along with the total and average population.

Epidemic of	No. of villages infected	Total population of villages infected	Average population of villages infected
1903—1904	57	149,524	2623
1904—1905	285	493,145	1730
1905—1906	30	117,631	3921
1906—1907	249	471,670	1894

TABLE LV.

Showing the number of villages in Rohtak District with the total and average population infected in no, one, two, three and four epidemics.

	No. of villages	Total population of villages	Average population of villages
Never infected	145	63,051	435
Infected in one epidemic	138	109,491	793
Infected in two epidemics	172	270,986	1575
Infected in three epidemics	37	128,669	3478
Infected in four epidemics	7	48,625	6946
Total	499	620,822	1244

TABLE LVI.

Showing the number of villages in Mozuffarnagar District infected each epidemic, with their mean population.

No. and year of epidemic	No. of villages infected	Total population of villages infected	Mean population of villages infected
1st 1902—03	25	75,341	3014
2nd 1903—04	130	257,091	1978
3rd 1904—05	313	532,941	1703
4th 1905—06	69	183,270	2656
5th 1906—07	579	744,948	1286

TABLE LVII.

Showing the number of villages in Mozuffarnagar District with their mean population infected in no epidemic, in only one epidemic, in any two, in any three, in any four and in all five epidemics.

	No. of villages	Total population of villages	Mean population of villages
No epidemic	334	127,217	381
One epidemic	301	211,861	704
Two epidemics	226	284,843	1260
Three epidemics	86	187,300	2178
Four epidemics	25	83,231	3329
Five epidemics	1	23,444	23,444
Total	973	917,896	942

TABLE LVIII.

Showing the number of villages in Amritsar District infected each epidemic, with their mean population.

No. and year of epidemic	No. of villages infected	Total population of villages infected	Mean population of villages infected
1st 1901—02	63	269,467	4277
2nd 1902—03	506	734,753	1452
3rd 1903—04	445	682,138	1533
4th 1904—05	669	869,853	1300
5th 1905—06	276	510,464	1849
6th 1906—07	604	813,116	1346

TABLE LIX.

Showing the number of villages in Amritsar District with their mean population, infected in no epidemic, in only one, in any two, three, four, five, and in all six epidemics.

Infected in	No. of villages	Total population of villages	Mean population of villages
No epidemic	155	39,225	253
One epidemic	183	83,779	458
Two epidemics	211	125,899	597
Three epidemics	230	210,281	914
Four epidemics	169	206,547	1222
Five epidemics	93	155,971	1677
Six epidemics	21	217,888	10,376
Total	1062	1,039,590	978

TABLE LX.

Showing in Rohtak District for each Tehsil the number of villages and their percentage on the total villages infected in no epidemic, and in one, two, three and four epidemics.

	Rohtak Tehsil	Gohana Tehsil	Sampla Tehsil	Jhaggar Tehsil
Total no. of villages	109	79	124	187
Total population	195,423	140,682	160,262	124,455
Average population per village	1793	1781	1292	666
No. of villages never infected	21	6	21	97
Percent. of villages never infected	19·3	7·6	16·9	51·9
No. of villages inf. 1 epidemic	28	13	36	61
Percent. of villages inf. 1 epidemic	25·7	16·5	29	32·6
No. of villages inf. 2 epidemics	39	47	61	25
Percent. of villages inf. 2 epidemics	35·8	59·5	49·2	13·4
No. of villages inf. 3 epidemics	17	12	5	3
Percent. of villages inf. 3 epidemics	15·6	15·1	4	1·6
No. of villages inf. 4 epidemics	4	1	1	1
Percent. of villages inf. 4 epidemics	3·7	1·3	0·9	0·5

PART II. STATISTICAL ANALYSES OF DATA RESPECTING
EPIDEMICS OF PLAGUE IN THREE DISTRICTS
OF THE PUNJAB.

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First Report—*On the factors which influence the frequency of infection.*

It was decided to proceed with the analysis of the data collected by Major Lamb in the following stages:—

1. To determine whether the numbers of villages attacked in any one, two or more epidemics were in agreement with the numbers one would expect to find supposing the matter were merely one of chance.

2. In the event of such distributions not proving to be random, to attempt to account for the non-random character, paying special attention to the supposed importance of size of village in this connection.

3. In the event of the distribution being non-random, and its character not being explained by variations in the populations, to investigate the matter further.

4. To consider and report upon the variations in percentages of deaths which were manifest even in villages of approximately the same size.

Since many of the points referred to are of considerable importance and their statistical treatment will be a somewhat lengthy process, it will make for clearness and avoid unnecessary delay if I embody my results in a series of separate reports. In the present communication, I shall deal with factors which influence the recurrence of infections, in so far as I have been able to reach statistical results. I desire also to remark that I have attempted on this occasion, and shall endeavour in any subsequent communications, to explain intelligibly the analytical methods I employ. I do not mean by this that I propose to discuss the somewhat difficult mathematical considerations from which the methods in question were, as a matter of history, derived; but merely

that I hope to furnish sufficient explanation to place the reader in a position to judge for himself whether the processes employed and the conclusions stated are, or are not, justifiable.

The first point to be observed is that one must adopt some standard as to what constitutes an infected village. When, for instance, only a few cases of plague have been found in a large village, it may be that these have been imported and are no true indications of local morbidity. To decide this point is not the duty of a statistician, since it involves special knowledge only possessed by those familiar with local conditions. I have, for the purposes of the present inquiry, been instructed by those who have this knowledge to regard as an infected village any village from which a plague death has been reported; the effect of modifying this criterion may be matter for subsequent inquiry.

At the outset, it is apparent that the size of the village is an important factor in the likelihood of its being infected. This is almost axiomatic on any theory of aetiology and is abundantly demonstrated by the statistics of which Table I give merely a selection.

The next point is to consider whether this factor is alone responsible for the selective grouping of villages affected in various combinations of epidemics, but before examining this we must consider the analytical process to be used.

The problem is of the following nature—There are a villages in a district and plague infections have been reported in n different epidemics, c_1 in the first, c_2 in the second, and so on, up to c_n . We also know that a_n villages have been infected in all epidemics, a_{n-1} in $n-1$ of the epidemics, and so on. Supposing that the fact of being infected in any one year is quite independent of the fact of being affected in any other year, is the number of actual recurrences in agreement with what we should expect?

This problem may be approached in the following way. The chance of being affected in the first epidemic is $\frac{c_1}{a}$, of being affected in the second epidemic $\frac{c_2}{a}$, and so on. Therefore the chance of being affected in all epidemics is $\frac{c_1 c_2 c_3 \dots c_n}{a^n}$ and the total number which should be so infected is $a \times \frac{c_1 c_2 c_3 \dots c_n}{a^n}$. Thus the number which would be infected in the first three epidemics is $a \times \frac{c_1 c_2 c_3 d_4 d_5 \dots d_n}{a^n}$ where

$$d_4 = a - c_4 \quad d_5 = a - c_5 \quad \text{etc.}$$

And we shall find the number infected in any three epidemics by taking a times the sum of all such expressions as

$$\frac{c_1 c_2 c_3 d_4 d_5 d_6 \dots d_n}{a_n}.$$

In this way one obtains the most probable number of infections for each possible combination of epidemics and we next require some method of testing the agreement between the observed numbers and the calculated ones. Of the various tests which have been proposed the most satisfactory is that devised by Karl Pearson¹ which can, for our purposes, be looked at in the following simple way.

Supposing the observed value in any case is x and the value expected, on any hypothesis which we desire to test, y . Then $\frac{(x-y)^2}{y}$ will be a measure of agreement independent of sign and its summed value $S \frac{(x-y)^2}{y}$, for all the classes we have formed will be a measure of the total agreement. The problem now reduces itself to the following—How many times, on the average, will the observed values in a series of events which are known to follow a given law of distribution differ from the expected values owing to random sampling so as to produce a specified arithmetical value of $S \frac{(x-y)^2}{y}$?

This has been answered by Pearson in the memoir referred to and a table constructed by W. Palin Elderton².

For instance, suppose that in a certain case $S \frac{(x-y)^2}{y}$, or as it is usually termed χ^2 , is equal to 10 and we find against this value for material grouped in the number of subdivisions used for our material, the fraction .8. This means that if the law be true we should get, on repeating our observations, as bad an agreement as or a worse agreement than that given by our material, eight times out of ten. In other words it is most likely that the theoretical law assumed really describes the observations.

What value of P (the .8 of my illustration is called P) should be taken as good evidence for the truth of one's hypothesis, is partly a

¹ "On the Criterion that a given System of Deviations from the Probable in the Case of a Correlated System of Variables is such that it can be reasonably supposed to have arisen from Random Sampling." K. Pearson, *Phil. Mag.*, 1900, Vol. L., p. 157.

² "Tables for Testing the Goodness of Fit of Theory to Observation." W. P. Elderton, *Biom.*, 1902, p. 155.

matter of individual judgment and partly depends on the nature of the material examined. I should be disposed to regard any value of P greater than .45 as good and greater than .25 as fair evidence in such problems as those we have to consider now. I do not propose to examine the mathematical justification of the method I have outlined; reference can be made by those readers who are interested in the subject to the memoir of Pearson which I have cited. It is, I hope, sufficiently clear that the process lends itself to our particular investigation. Some practical difficulties now deserve notice.

Although the calculation of the probable number of villages to be infected in any combination of epidemics is, as I have shown, merely a matter of simple arithmetic, yet the arithmetic becomes extremely laborious when the number of epidemics under review is even moderately large. Thus in Amritsar district there are six epidemics and the complete evaluation of the various combinations of villages affected never, once, twice etc., requires the determination of 64 distinct products each composed of six terms. Without a mechanical calculator this is an impracticable task and even with such help it is very tedious. It may be asked whether an approximation to the value cannot be obtained by some easier method and a way out of the difficulty might seem to be afforded by using a mean value. Thus if c_1, c_2, c_3 etc. were infected in each year we might determine the mean probability of infection

$$\frac{c_1 + c_2 + c_3 + \dots c_n}{na} = \text{say } \bar{p},$$

and, similarly, the mean probability of not being infected = say \bar{q} . Then the number which should be infected in any two years *e.g.* would be $a (\bar{p})^2 (\bar{q})^{n-2}$. The objections to this simplification are, I think, insuperable. The total number of epidemics under observation is absolutely small and the proportion of villages attacked from epidemic to epidemic, varies enormously. For instance, in Amritsar, out of 1062 villages—

62	were attacked in	1901—2
506	„ „	1902—3
445	„ „	1903—4
669	„ „	1904—5
276	„ „	1905—6
604	„ „	1906—7.

The probable error of a mean determined from so short and variable a series is large (for *normally distributed variables*, the probable error of the mean is $\cdot67449 \times \frac{\text{Standard Deviation}}{\sqrt{\text{Number of Observations}}}$).

The difference between the results obtained by calculating the probable numbers in both ways is appreciable as will be seen in Table II. I have therefore made all the calculations by the direct summation method already described. Although the process was very long it was rendered possible by the use of a large Brunsviga Calculator and all additions were checked with a Burroughs Adding Machine.

I shall give the complete working of one table, that for the whole Amritsar district. From the figures given above, we see that the chances of being infected in each year are:

$$p_1 = \cdot0583804, \quad p_2 = \cdot4764595, \quad p_3 = \cdot4190207, \\ p_4 = \cdot6299435, \quad p_5 = \cdot2598870, \quad p_6 = \cdot5687382,$$

and the corresponding chances of not being infected are:

$$q_1 = \cdot9416196, \quad q_2 = \cdot5235405, \quad q_3 = \cdot5809793, \\ q_4 = \cdot3700565, \quad q_5 = \cdot7401130, \quad q_6 = \cdot4312618.$$

With these values for the independent probabilities, we determine the expected number in each category. Thus the probable number affected five times is

$$1062 (p_1 p_2 p_3 p_4 p_5 q_6 + p_1 p_2 p_3 p_4 p_6 q_5 + p_1 p_2 p_3 p_5 p_6 q_4 + p_1 p_2 p_4 p_5 p_6 q_3 \\ + p_1 p_3 p_4 p_5 p_6 q_2 + p_2 p_3 p_4 p_5 p_6 q_1).$$

Finally we reach Table III the headings of which are explanatory. The value of P is so small that we can evidently not regard the distribution as a random one. Tables IV and V show similar results for the other two districts. Table III A gives the details for Amritsar.

We now come to the question whether the non-random character of these distributions is due to mixing together villages of very different sizes. I approached the problem in the following way. A series of groups was chosen, each containing villages falling within assigned limits of size. Each group was then treated in the manner just explained, the probability of infection in each epidemic being of course determined separately for each group. The results appear in Tables VI to XXV, the actual working details being omitted to save space. These tables merit somewhat close attention. In the first place it is obvious that the majority exhibit a much closer agreement between theory and

observation than could be discerned in the general Tables III to V, but an objection will immediately occur to the reader. Tables VI to XXV are sub-samples and consequently include far fewer observations than figure in the Tables III to V, is this the cause of the better agreement?

It is clear that the test of agreement which I have employed gives an answer which in some measure depends on the absolute size of the experience. Thus our criterion is the value of

$$S \frac{(x-y)^2}{y} = \chi^2.$$

If we multiply x and y by a constant k this becomes:

$$S \frac{k^2 (x-y)^2}{ky},$$

or

$$S \frac{k (x-y)^2}{y}.$$

Hence if k is a proper fraction χ^2 is diminished and the apparent goodness of fit increased. This is, of course, in accordance with the demands of common sense. A deviation of, say, ten per cent. from the theoretical value when we examine, say, a sample of 100 would not be regarded as making so much against the theory as a ten per cent. difference when the sample was one of 1000.

Now it seemed to me that one could form a reasonable opinion as to whether the higher values of P in some of the groups are merely due to reduction in absolute size and not to reduction in the relative discrepancy between theory and practice, quite simply. I have taken each table of totals (III—V) and determined the value which P would take if the relations between observed and calculated values were not disturbed but the value of χ^2 reduced to what would be given by smaller total numbers of observations.

In the case of Amritsar, the values of χ^2 reduced to 153, 129, 390 observations are still so large as to give values of P less than one in a billion; even with a total of 45 (the smallest Amritsar Group) and using five classes instead of six, P only rises to .0001. The Mozuffarnagar total reduced to 72, 82, 141 and 161 observations also gives P less than one in a million. Rohtak gives $P = .04$ when the total is reduced to 44 and $P = .01$ for 60 observations. While I admit the test to be a rough one, I can see no valid objection to it. I hold it to be certainly true that the improved agreement between theory and practice shown in many of the Tables VI—XXV is not to any serious

extent dependent on the reduction in numbers, as compared with Tables III—V alone. We are now in a position to assert that size of population is an important factor in determining whether a village is likely to be attacked by plague, and that elimination of this factor tends to bring the number of recurrent infections into agreement with a chance distribution, but a variety of collateral questions are raised by the analytical results. To begin with, there is not only a great difference in the results from the several districts, but between the returns for groups of the same district. Why is this? Taking the second point first, the most obvious explanation would seem to be that the groups are arbitrary and that the variations in size within each are not the same. There is no real reason to think that absolute size within the group has anything to do with the matter, because the *a priori* chance of being infected has been calculated separately for each group; it could only be a question of relative variation. I determined the mean population of each group, the mean square deviation from the mean, its square root (the Standard Deviation) and the percentage that the standard deviation was of the mean (the Coefficient of Variation). These constants are shown in Tables VI to XXV and are collected together in Table XXVI. Taken as a whole, there appears to be a *slight* tendency for the higher values of P to be associated with smaller coefficients of variation and this was to some extent confirmed by determining the correlation between the value of P and the size of the coefficients of variation. I cannot, however, attribute any real importance to these findings. The figures marked with an asterisk are hardly comparable with the others since the variation depends mainly on the inclusion of a few very large or very small villages or towns. But if we exclude these, the variation is rarely in excess of 10. Now the census from which the populations were obtained was taken in 1901 and I understand that a variety of local circumstances prevent the census returns being very accurate (as a basis for estimating the present population). I was advised that one could not safely regard the error as being much less than ten per cent. In other words the variation within the groups is of the same order as the error in the estimates of population and cannot serve as a basis for valid statistical deductions. I am, therefore, of opinion that we cannot attribute the differences in the values of P to this factor. I must not of course be understood to mean that differences in size-variation have not played a part, but merely as stating that we have no reliable evidence that such is the case.

A contributory influence of appreciably greater weight is the difference in number of villages within each group, especially as in some of the smaller groups fewer subdivisions were made owing to the calculated numbers of infections in five and six epidemics being very small. I have tested this in the same way as in comparing totals with groups (*vid. supr.*). Table XI gives $P = \cdot 07$ for 129 villages and this rises to $\cdot 57$ for 44 observations. Table X gives $P = \cdot 148$ when we reduce the number of categories from 6 to 5 and the total to 44. Similarly, Table IX reduces to $P = \cdot 24$ and Table VIII to $P = \cdot 275$. Using the Mozuffarnagar results we find, reducing to a total of 44 and five categories (which enables us to compare with the Rohtak figures), Table XVI gives $P = \cdot 44$, Table XIV, $P = \cdot 08$, Table XV, $P = \cdot 21$.

These results show quite clearly that the size of the group and the number of sub-classes, whether 7, 6, or 5, employed, have had a considerable share in causing diversity within each district. They also show that the greatly improved fit among the Rohtak Groups as compared with Mozuffarnagar and especially Amritsar, is *partly*, but only partly accounted for, by the differences mentioned. We have seen that in one group only out of the Amritsar set have we been able to bring up the goodness of fit to the general level of the Rohtak returns. The same remark applies but with appreciably less force to a comparison between Mozuffarnagar and Rohtak. Table XXVIII contains all the Rohtak groups which were calculated in five classes reduced to a total of 44 and all the returns from the other districts reduced to the same dimensions. It will be found to support the preceding observations to the effect that Amritsar shows definitely less good agreement with expectation than do the other districts. The averages for each district are simple means formed without weighting with the numbers which actually appeared in each group; it is hardly possible to give truly comparable means because of the difficulty of assigning proper weights.

It therefore results, I think, that while careful allowance is to be made for the various sources of fallacy which are involved in attempting such comparisons as these, possibilities of error which I have not—consciously at least—minimised, there is an appreciable difference between the returns for any one district within that district and a still more appreciable difference between the returns for Amritsar on the one side and Rohtak on the other.

In the case of Rohtak, the general run of values for P is so good that we seem entitled to conclude that size of village has been by far the most important factor in producing the want of agreement which

is seen in the general Table. Other factors may have contributed to the results but their importance would seem to have been relatively small.

In Mozuffarnagar more hesitation is appropriate, but the fit in some important cases is so good that we are still justified in assigning the pre-eminence to the size factor¹. In Amritsar, although certainly one and possibly two examples prove that size has been of considerable importance, yet the general trend of the tables seem to force one to the conclusion that something else has had a special influence not to be discerned in the other districts. What this something else is, I do not see my way to deciding on the strength of the information before me. A study of the maps and of the extremely interesting written descriptions with which I was furnished appear to show certain differences. Rohtak appears to be less thickly peopled than Amritsar²; Amritsar is somewhat better served by means of communication and possesses much larger and more important towns. Nothing which I have been able to find in the report of the Commissioners or in such descriptions of plague epidemiology as I have consulted, has suggested anything capable of statistical analysis with respect to these points.

The following ideas have occurred to me. Amritsar is much the largest city in the three districts and its intercourse with other towns and villages in the district must be great even out of proportion to its size, since it is the centre of important administrative business and is of religious interest to the Sikhs. The main lines of migration to and from the city are presumably not random, just as we find in England that of two apparently equally convenient highways between large towns one is much the more often traversed. Villages lying along the habitual lines of travel might be specially prone to importation of sources of infection irrespective of their size or other local peculiarities. The difficulty of testing this statistically lies in the fact that even in Amritsar size plays a part in the recurrence or non-recurrence of infections, but still the matter might be tested and I will do so³, unless

¹ It may, perhaps, be asked why, in view of Table XXVIII, I class Mozuffarnagar rather with Rohtak than with Amritsar. The answer is that, apart from the rough nature of the test which Table XXVIII exemplifies, certain of the groups in Mozuffarnagar, notably Table XII with a relatively large number of included villages, exhibit a fairly close agreement between expectation and observation. In all the circumstances, I think this fact should have weight. The reader will of course draw his own conclusions.

² Rohtak 3 per acre, Amritsar 1 per acre. I have no record of the area of Mozuffarnagar District.

³ A preliminary analysis shows that the Amritsar villages within two miles of the line of railway are not a random sample of the total in respect to plague recurrence.

those whose practical knowledge of the aetiological problem is far greater than mine, decide that the idea is baseless.

Another point which might be worth consideration is the possibility that the greater percentage of Sikh inhabitants, who are, I understand, more numerous in the Amritsar district than elsewhere, is of some significance.

One other conclusion seems to be supported by the statistical evidence. In investigating the arithmetical cause of the poor agreement between expectation and observation in Amritsar district, I was struck by the fact that the main discrepancy was often due to the calculated number of never-infected villages falling short of the observed number. To see whether this were really so, I constructed Table XXVII. It will be seen that in the majority of cases, notably in Amritsar, the observed excess in the first group has been largely responsible for the poor fit. It might be hastily assumed that this is due to taking an arbitrary standard of plague infection, viz. the occurrence of a single death. This is not necessarily or even probably the case. The number of such villages is not very large and were it large the *a priori* probability of non-infection would be greatly increased by including the villages with one or two deaths in the never-infected class. This would disturb the balance altogether and might conceivably make the agreement even worse. This is a point I can test, if the Committee so desire; as the case stands, I am disposed to conclude that certain villages, for reasons which do not appear in the mere statistics, are peculiarly difficult to infect.

Since the question as to whether a village infected in one year is, *ipso facto*, more likely to become infected in the next following year has a special bearing on the problem of importation as contrasted with recrudescence, I have investigated the point separately in the following way.

Six groups of Amritsar villages were chosen, viz.

- | | | |
|-----|-------------------------|------------|
| (1) | Villages of populations | 1200—1400. |
| (2) | " " | 1000—1200. |
| (3) | " " | 800 —1000. |
| (4) | " " | 500 — 700. |
| (5) | " " | 400 — 500. |

The numbers infected in the years 1904, 1905, 1906, 1907, were ascertained and then the numbers expected to be attacked in any pair of consecutive years were calculated on the assumption of equal incidence within the groups.

The actual numbers were then compared with the calculated figures for each group and each pair of years. The goodness of agreement was then measured by the method used in other sections of this report. The results are collected in Table XXIX.

These results merit careful attention. It will be noticed that for the years 1904—5 and 1905—6, the agreement as measured by the value of P is extremely close, very fair for 1903—4, and moderate or poor (in view of the total number of observations) for 1906—7. The agreement obtained when the totals are used and grouped in years may be considered moderate.

On looking at the details, we notice that the agreement is, in general, worse in the groups within which the relative variations in size are largest. Thus the groups of big villages agree quite well in all cases but one and the poor agreement in the table for 1906—7 is mainly due to the group 500—700, while the fit for 1903—4 is appreciably diminished by the same group. It must, however, be carefully observed that this particular group is the largest and should have the most weight assigned to it. It must also be noticed that the actual numbers are, in the majority of cases, larger than the calculated ones and that where the agreement is best the likelihood of failure is least.

Let us next consider what sort of results we should expect to get, if we were to adopt an hypothesis as to epidemic origins.

If we hold that a plague outbreak depends on the recrudescence or bursting into activity of infective agencies left over from the last epidemic, then the following deductions are, in my opinion, inevitable. Only villages which have been attacked previously, in some shape or form, can *ex hypothesi* be attacked again. All those which have been so evidently infected as to report at least one death will be eligible for reinfection. In addition there will be some villages so slightly affected or placed under such unfavourable circumstances for the obtaining of exact information that they do not figure in our returns but which are eligible for attack in the following year. We should not, therefore, be surprised to find that *a very small proportion* of the villages which showed cases in any one year had not been *ostensibly* affected in the previous year. Unless, however, we assume that in two consecutive outbreaks the first is always much milder than the second, an assumption without evidence in its favour, the magnitude of this error cannot be sufficient to vitiate the general statement that the brunt of the second outbreak must be borne by villages affected in the first year. One would therefore expect a marked discrepancy between the number of

villages twice infected and the number calculated on the assumption that the infections in two years are independent events. Of course when a very large proportion of the villages is infected in both years, the possibility of a discrepancy is diminished. For instance, suppose in a group of 100 villages, 90 are infected in one year and 90 again in the second, we should expect as a matter of chance that 81 would be infected in both years and on the recrudescence hypothesis we could not have many more than 90 affected twice. Such a discrepancy as this, when the numbers are small, would be consistent with a high value of P . While these considerations must be well weighed, it seems to me that sufficient margin exists in the groups of villages and sequences of epidemics analysed in the table to have afforded scope for the numbers of twice affected villages to exceed the calculated numbers more definitely than was actually the case. To make this clear Table XXX was prepared.

This table shows the percentage of villages in each group affected in each epidemic, which did not return cases in the previous epidemic. This shows how large a proportion of villages affected in any one year had not returned cases in the previous year (*vide supra* p. 363).

It therefore seems clear that the agreement between the chance distribution and the actual one is closer than we should expect were the recrudescence hypothesis actually to express an epidemiological truth.

On the other hand, if we adopt the hypothesis of importation examined in the first part of this report, we should not expect to find an absolute agreement between chance and observation, we should still expect the fact of previous infection to have some importance, for the following reasons.

What the factors may be which determine importation has not yet been rendered precise. That habitual lines of travel, *e.g.* railways, are influential is suggested by many facts, especially the maps published by Nathan in his report of the 1896 outbreak and an analysis of the plague history of Amritsar villages within two miles of a railroad which I shall publish in a subsequent report. But, in any case, it may be regarded as certain that the influences of whatever nature tend to act in the same direction for fairly long periods of time, that if any village is favourably situated for importation in 1906 it is likely to be favourably situated again in 1907. Hence, we should expect to find that villages which have been infected once are on the whole more likely to be infected in the following epidemic than villages taken at random. But

since these circumstances are external to the village, and not part and parcel of the infection itself, they *may* be changed. A virulent outbreak in one year may deter visitors from approaching it in the following plague season, sanitary measures may be enacted and enforced, these and a thousand other circumstances dependent on the mutability of human actions might tend to weaken or even reverse the presumption created by a first infection. The conclusion is that while on the recrudescence theory we should expect to find a strong predisposition to reinfection in the case of villages once attacked, on the theory of reimportation, the influence of a first infection should be slighter and variable. The statistical evidence is, I think, more consistent with the second alternative.

In view of the smallness of the experience—from a statistical point of view—and the non-uniformity of the results, I am not justified in asserting that the evidence here adduced disproves the recrudescence theory. It does, however, somewhat strengthen the case against it. When this evidence is combined with that advanced in the first part of the present report, the case becomes, in my opinion, a rather strong one.

The broad conclusions which may be drawn from the present analysis are, I think, the following:—

(1) In none of the three districts can the total distribution of villages into classes showing no, one, two, etc. infections possibly be regarded as a chance event.

(2) In Rohtak, grouping villages of approximately the same size together and considering these groups separately, markedly diminishes the non-random character of the distribution. The agreement between the numbers in each group which were 0, 1, 2, 3 or 4 times affected and the numbers calculated on the assumption that the distribution was a chance one is in every case fair and in some excellent. This agreement is exaggerated by the relatively small numbers of villages in the groups and paucity of epidemics in comparison with the other districts, but when allowance is made for these circumstances, it is still evident that Rohtak yields better agreements than the other districts, in particular much better agreements than in the Amritsar groups.

(3) The Mozuffarnagar groups also show marked improvement as compared with the total for that district, an improvement not accounted for by the smaller size of the groups as compared with the total. They do not yield such uniformly good results as Rohtak but some are so excellent as to warrant one in concluding that size of villages in

Mozuffarnagar as in Rohtak is much the most important cause of the discrepancy found in the table for the whole district.

(4) Grouping in Amritsar has improved the agreement sufficiently to warrant the assertion that here also size of village has been of importance. The results, however, are not generally good and cannot, by allowing for size and number of classes, be sufficiently improved to justify us in regarding size of village as having played so decisive a part as in the other districts.

(5) Nothing in the statistical evidence affords a satisfactory explanation of this difference which may, however, profitably be made the object of further statistical inquiry.

(6) Differences in the numbers of villages within each group partly account for the differences in goodness of agreement within each set of groups, but not entirely.

(7) There is not sufficient statistical evidence that relative variations in size within the groups account for this divergence.

(8) There is no good reason to think that the fact of a village having been infected in one epidemic renders it more likely to be infected in the following epidemic or less likely to be so infected than any other village.

It may perhaps seem to the reader that the analysis here presented is not fine enough to do justice to the valuable material. While I fully recognise that the present data in all probability constitute the most valuable and complete statistical materials which have ever been collected for the study of plague, still, possible sources of error which have been pointed out to me, especially by Major Lamb, dispose me to think that an ostensibly more refined analysis might be misleading. The conclusions herein presented may not be altogether without interest and value.

TABLE I.

To show the increase in percentage of infected villages as the population increases.

[Cf. the Tables LIV—LIX *supra*.]

Mean population of Groups	Percentage never infected	Percentage 1 infection	Percentage 2 infections	Percentage 3 infections	Percentage 4 or more infections
Rohtak.					
92	80.00	20.00	0	0	0
257	51.22	31.71	17.07	0	0
352	50.00	33.33	14.67	0	0
603	30.65	32.26	32.26	4.84	0
784	20.93	39.53	34.88	4.65	0
1059	15.25	33.90	45.76	1.69	3.39
1465	6.82	25.00	61.36	6.82	0
2373	5.13	15.38	64.10	15.38	0
Mozuffarnagar.					
404	34.76	45.12	17.63	1.22	1.22
606	25.53	45.39	23.40	5.67	0
917	21.58	28.06	38.13	10.79	1.44
1247	9.76	23.17	45.12	18.29	3.66
1746	6.94	23.61	40.28	23.61	5.56
4137	4.49	15.73	33.70	29.21	16.85
Amritsar.					
175	37.78	24.44	28.89	4.44	4.44
276	24.53	30.19	18.87	20.75	5.66
308	21.88	33.13	23.75	15.00	6.25
708	7.44	20.51	22.05	29.49	20.51
1257	1.96	7.19	11.76	27.45	51.63
3837	0.78	2.33	4.65	18.60	73.64

TABLE II.

To show the difference between the results obtained by the direct method of calculation used in the other tables and the values obtained when a mean value for the probability of infection is used.

Amritsar District.		
Number of epidemics	Villages infected (calculated numbers)	
	Direct method	Mean method
0	36	48·5
1	182	195·8
2	351	329·1
3	323	295·1
4	143	148·8
5	26	40
6	1	4·5

TABLE III.

Numbers of villages affected in various combinations of epidemics compared with the expected numbers.

Amritsar District.					
Times affected	Actual number of villages	Calculated number of villages	Difference	Square of the difference	Square of the difference divided by the calculated number
0	155	36	+ 119	14161	393·36
1	183	182	+ 1	1	·01
2	211	351	− 140	19600	55·84
3	230	323	− 93	8649	26·78
4	169	143	+ 26	676	4·73
5	93	26	+ 67	4489	172·65
6	21	1	+ 20	400	400
	1062	1062			1053·37 = χ^2

P corresponding to $\chi^2=1053\cdot37$ is too small to be tabled in Elderton's Table. Calculation from the Subsidiary Table shows *P* to be much less than one in a billion. In other words, if the distribution is really a chance one, we should get so bad an agreement on the average less than once in a billion trials. The distribution can hardly, therefore, be regarded as a chance one.

TABLE III A.

Showing the details for various years.

Amritsar District.

Number of villages affected in various epidemics and combinations of epidemics together with the calculated probable numbers.

	Actual number	Probable number		Actual number	Probable number
1901	1	2.2	1902 & 4 & 5	10	19.5
1902	34	32.7	1902 & 4 & 6	79	73.4
1903	26	25.9	1902 & 5 & 6	4	15.1
1904	60	61.1	1903 & 4 & 5	5	15.5
1905	3	12.6	1903 & 4 & 6	44	58.2
1906	59	47.4	1903 & 5 & 6	6	12.0
	183	181.9	1904 & 5 & 6	25	28.3
				230	322.6
1901 & 2	1	2.0	1901 & 2 & 3 & 4	1	2.5
1901 & 3	1	1.6	1901 & 3 & 4 & 5	1	1.0
1901 & 4	2	3.8	1901 & 2 & 4 & 5	0	1.2
1901 & 5	1	0.8	1901 & 2 & 3 & 5	0	0.5
1901 & 6	1	2.9	1901 & 2 & 3 & 6	1	1.9
1902 & 3	15	23.6	1901 & 2 & 4 & 6	2	4.5
1902 & 4	30	55.6	1901 & 2 & 5 & 6	0	0.9
1902 & 5	6	11.5	1901 & 3 & 5 & 6	0	0.7
1902 & 6	22	43.1	1903 & 4 & 5 & 6	23	20.4
1903 & 4	23	44.1	1903 & 2 & 5 & 6	7	10.9
1903 & 5	9	9.1	1901 & 3 & 4 & 6	4	3.6
1903 & 6	13	34.2	1902 & 4 & 5 & 6	30	25.8
1904 & 5	16	21.5	1901 & 4 & 5 & 6	1	1.7
1904 & 6	65	80.7	1902 & 3 & 4 & 6	82	52.9
1905 & 6	6	16.6	1902 & 3 & 4 & 5	18	14.1
	211	351.1		170 (169)*	142.6
1901 & 2 & 3	1	1.5	1901 & 2 & 3 & 4 & 5	2	0.9
1901 & 2 & 4	0	3.5	1901 & 2 & 3 & 4 & 6	12	3.3
1901 & 2 & 5	0	0.7	1901 & 3 & 4 & 5 & 6	1	1.3
1901 & 2 & 6	0	2.7	1901 & 2 & 4 & 5 & 6	2	1.6
1901 & 3 & 6	3	2.1	1902 & 3 & 4 & 5 & 6	79	18.6
1901 & 4 & 6	2	5.0	1901 & 2 & 3 & 5 & 6	0	0.7
1901 & 5 & 6	0	1.0		96 (93)*	26.4
1901 & 3 & 5	0	0.6	1901 & 2 & 3 & 4 & 5 & 6	21	1.2
1901 & 3 & 4	0	2.7	Never affected	155	36.0
1901 & 4 & 5	0	1.3	Totals	1066 (1062)	1061.8
1902 & 3 & 6	14	31.1			
1902 & 3 & 4	33	40.1			
1902 & 3 & 5	4	8.3			

* The figures in brackets are those which appear in the MS. summary handed me and I used them for calculation. They do not agree exactly with the returns in the data papers, but the difference is quite unimportant.

TABLE IV.

MOZUFFARNAGAR DISTRICT.

Numbers of villages affected in various combinations of epidemics compared with the expected numbers.

Times affected	Actual number	Calculated number	Difference	Square of the difference	Square of the difference divided by the calculated number
0	334	209·60	+ 124·4	15475·36	73·832
1	301	461·268	− 160·268	256858·3182	556·853
2	226	254·486	− 28·486	811·4522	3·189
3	86	44·866	+ 41·134	1692·00596	37·712
4	25	2·734	+ 23·221	539·2148	194·032
5	1	·045			
	973	972·999			865·61880

P less than 1 in a billion.

TABLE V.

ROHTAK DISTRICT.

Numbers of villages affected in various combinations of epidemics compared with the expected numbers.

Times affected	Actual number	Calculated number	Difference	Square of the difference	Square of the difference divided by the calculated number
0	145	89·26	+ 55·74	3106·9476	34·808
1	138	225·00	− 87·00	7569·0	33·640
2	172	159·22	+ 12·78	163·3284	1·026
3	37	24·56	+ 12·44	154·7536	6·301
4	7	0·98	+ 6·02	36·2404	36·980
	499	499·02			112·755

P less than 1 in a billion.

TABLE VI.

AMRITSAR VILLAGES OF POPULATION BETWEEN 150 AND 200.

Comparison of actual and probable numbers affected.

Number of attacks	Actual numbers	Probable numbers
0	17	11·82
1	11	18·50
2	13	11·10
3	2	3·15
4	1	0·46
5	1	
	45	45·03

P = ·156.

Mean Population 175, Standard Deviation 13·94, Coefficient of Variation 7·97.

TABLE VII.

AMRITSAR VILLAGES OF POPULATION BETWEEN 250 AND 300.

Comparison of actual and expected number of attacks.

[One village only was affected in the first year of plague so that for convenience of calculation this year has been regarded as free.]

Number of attacks	Actual number of villages affected	Expected number	
0	13	8	
1	16	19.17	
2	10	17.21	
3	11	7.16	
4	3	1.36	} 1.456
5	0	.096	
	<hr/> 53	<hr/> 52.996	

 $P = .035.$

Mean Population 276, Standard Deviation 14.16, Coefficient of Variation 5.13.

TABLE VIII.

AMRITSAR VILLAGES OF POPULATION BETWEEN 200 AND 400.

Comparison of actual and calculated numbers attacked in various epidemics.

Number of epidemics	Actual number	Expected number	
0	35	23.65	
1	53	58.29	
2	38	52.18	
3	24	21.40	
4	6	3.89	} 4.08
5	4	.18	
6	0	.01	
	<hr/> 160	<hr/> 159.6	

 $P = .001.$

Mean Population 308, Standard Deviation 62.94, Coefficient of Variation 20.41.

TABLE IX.

AMRITSAR VILLAGES OF POPULATION BETWEEN 400 AND 1000.

Comparison of expected attacks with actual attacks.

Number of epidemics	Observed	Expected	
0	29	11.9	
1	80	64.61	
2	86	130.06	
3	115	121.49	
4	63	52.77	
5	16	8.78	} 9.01
6	1	0.23	
	<hr/> 390	<hr/> 389.84	

 P less than .0000001.

Mean Population 708, Standard Deviation 177.97, Coefficient of Variation 25.13.

TABLE X.

AMRITSAR VILLAGES OF POPULATION BETWEEN 1050 AND 1600.

Comparison of numbers actually affected with expectation.

Number of epidemics	Actual number affected	Expected number
0	3	0.32
1	11	4.38
2	18	21.64
3	42	49.27
4	46	52.59
5	27	22.56
6	6	2.19
	153	152.95
	$P = .000031.$	

Mean Population 1257, Standard Deviation 172.86, Coefficient of Variation 13.75.

TABLE XI.

AMRITSAR VILLAGES OF POPULATION GREATER THAN 1600.

Comparison of numbers affected in various epidemics with the calculated numbers.

Number of epidemics	Actually affected	Expected number
0	1	0.015
1	3	0.15
2	6	5.26
3	24	25.29
4	42	53.29
5	44	39.15
6	9	5.92
	129	129.08
	$P = .07.$	

Mean Population 3837, Standard Deviation 14065.42, Coefficient of Variation 366.61.
 [This group includes Amritsar town.]

TABLE XII.

MOZUFFARNAGAR VILLAGES 350—500.

Comparison of actual numbers affected in various epidemics with expected numbers.

Number of attacks	Actual number	Expected number
0	57	50.87
1	74	82.90
2	29	27.63
3	2	2.54
4	2	0.06
	164	164.0
	$P = .48.$	

Mean Population 404, Standard Deviation 58.75, Coefficient of Variation 14.54.

TABLE XIII.

MOZUFFARNAGAR VILLAGES 500—750.

*Comparison of numbers actually affected in various epidemics
with expected numbers.*

Number of times affected	Actual number	Expected number
0	36	28.11
1	64	76.05
2	33	32.75
3	8	3.82
4	0	0.18
5	0	0.003
	<hr/> 141	<hr/> 140.91

$$P = .11.$$

Mean Population 606, Standard Deviation 64.57, Coefficient of Variation 10.65.

TABLE XIV.

MOZUFFARNAGAR VILLAGES BETWEEN 750 AND 1100.

Comparison of actual numbers of cases with expectation.

Number of attacks	Actual number of villages	Expected number
0	30	15.12
1	39	62.74
2	53	48.97
3	15	11.43
4	2	0.81
5	0	0.01
	<hr/> 139	<hr/> 139.08

} .82

$$P = .000007.$$

Mean Population 917, Standard Deviation 100.61, Coefficient of Variation 10.98.

TABLE XV.

MOZUFFARNAGAR VILLAGES BETWEEN 1100 AND 1450.

*Comparison of numbers actually affected in different epidemics with
expected numbers.*

Number of times affected	Actual number affected	Expected number
0	8	3.02
1	19	25.12
2	37	38.51
3	15	13.79
4	3	1.61
5	0	0.05
	<hr/> 82	<hr/> 82.1

} 1.66

$$P = .03.$$

Mean Population 1247, Standard Deviation 100.21, Coefficient of Variation 7.73.

TABLE XVI.

MOZUFFARNAGAR VILLAGES BETWEEN 1400 AND 2200.

Comparison of numbers actually affected in various epidemics with the expected numbers.

Number of epidemics	Actual number	Expected number
0	5	2·19
1	17	17·78
2	29	34·14
3	17	15·71
4	4	2·12
5	0	0·07
	<hr/> 72	<hr/> 72·01

$P = \cdot 28$.

Mean Population 1746, Standard Deviation 188·85, Coefficient of Variation 10·81.

TABLE XVII.

MOZUFFARNAGAR VILLAGES OVER 2000.

Comparison of numbers actually affected in various epidemics with calculated numbers.

Number of epidemics	Number actually affected	Calculated number
0	4	0·94
1	14	8·39
2	30	36·56
3	26	30·47
4	14	12·08
5	1	0·57
	<hr/> 89	<hr/> 89·01

$P = \cdot 0064$.

Mean Population 4137, Standard Deviation 3146·29, Coefficient of Variation 76·05.

[Includes villages of population 11,563, 19,304, 23,444.]

TABLE XVIII.

ROHTAK VILLAGES 1—200.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number	Expected number
0	40	40·5
1	10	9·0
2	0	0·5
	<hr/> 50	<hr/> 50·0

$P = \cdot 76$.

Mean Population 92, Standard Deviation 68·96, Coefficient of Variation 74·81.

TABLE XIX.

ROHTAK VILLAGES 200—300.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number	Expected number
0	21	18·53
1	13	18·22
2	7	3·98
3	0	0·27
4	0	0·00
	<hr/> 41	<hr/> 41·00

 $P = \cdot 36.$

Mean Population 257, Standard Deviation 27·81, Coefficient of Variation 10·83.

TABLE XX.

ROHTAK VILLAGES BETWEEN 300 AND 400.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Actual number	Expected number
0	24	22·44
1	16	20·47
2	8	4·75
3	0	0·34
4	0	0·05
	<hr/> 48	<hr/> 48·05

 $P = \cdot 30.$

Mean Population 352, Standard Deviation 30·34, Coefficient of variation 8·62.

TABLE XXI.

ROHTAK VILLAGES 500—700.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number	Expected number
0	19	14·33
1	20	28·69
2	20	16·69
3	3	2·27
4	0	0·03
	<hr/> 62	<hr/> 62·01

 $P = \cdot 28.$

Mean Population 603, Standard Deviation 57·08, Coefficient of Variation 9·46.

TABLE XXII.

ROHTAK VILLAGES 700—900.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number	Expected number
0	9	7·40
1	17	19·76
2	15	14·29
3	2	1·56
	43	43·01
	$P = \cdot 82.$	

Mean Population 784·23, Standard Deviation 57·36, Coefficient of Variation 7·31.

TABLE XXIII.

ROHTAK VILLAGES 950—1200.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number	Expected number
0	9	5·35
1	20	25·67
2	27	24·69
3	1	3·21
4	2	0·08
	59	59·0
	$P = \cdot 26.$	

Mean Population 1059, Standard Deviation 87·18, Coefficient of Variation 8·24.

TABLE XXIV.

ROHTAK VILLAGES POPULATION 1400—1700.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Actual number	Expected number
0	3	2·0
1	11	14·12
2	27	23·89
3	3	3·86
4	0	0·13
	44	44·00
	$P = \cdot 75.$	

Mean Population 1465, Standard Deviation 130·92, Coefficient of Variation 8·94.

TABLE XXV.

ROHTAK 2000—2950.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Actual number	Expected number
0	2	0·80
1	6	8·78
2	25	23·40
3	6	5·67
4	0	0·36
	<hr/> 39	<hr/> 39·01

 $P = \cdot 53.$

Mean Population 2373, Standard Deviation 230·89, Coefficient of Variation 9·73.

TABLE XXVI.

Relation between Goodness of Fit and Variation in Size.

No. in Group	Average Population	Coefficient of Variation	P
ROHTAK.			
*50	92	68·96	·76
41	257	10·83	·36
48	352	8·62	·30
62	603	9·46	·28
43	784	7·31	·82
59	1059	8·24	·26
44	1465	8·94	·75
39	2373	9·73	·53
MOZUFFARNAGAR.			
164	404	14·54	·48
141	606	10·65	·11
139	917	10·98	·000
82	1247	7·73	·03
72	1746	10·81	·28
*89	4137	76·05	·006
AMRITSAR.			
45	175	7·97	·156
53	276	5·13	·035
160	308	20·41	·001
*390	708	25·13	·000
153	1257	13·75	·000
*129	3837	366·61	·07

TABLE XXVII.

Showing the influence of the non-infected villages on the Goodness of Fit.

No. of villages in the group	Percentage of total number in the group which should not be infected	Actual difference between calculated and observed number	Percentage contribution of this difference to the value of χ^2	Fit
ROHTAK.				
39	2.05	+ 1.2	56.80	.53
62	23.01	+ 4.68	30.02	.28
48	46.71	+ 1.56	2.93	.30
43	17.21	+ 1.60	38.74	.82
41	45.19	+ 2.47	7.53	.36
44	4.55	+ 1.0	26.10	.75
59	9.07	+ 3.65	62.50	.26
50	81.00	- .5	1.00	.76
MOZUFFARNAGAR.				
82	3.68	+ 4.98	74.99	.03
139	10.88	+ 14.88	54.71	.00
72	3.04	+ 2.91	57.63	.28
164	31.02	+ 6.13	29.36	.48
141	19.94	+ 7.89	24.93	.11
89	1.06	+ 3.06	61.58	.01
AMRITSAR.				
153	3.07	+ 9.3	64.76	.00
390	3.05	+ 17.1	46.73	.00
53	15.09	+ 5.0	30.14	.04
45	26.27	+ 5.18	33.94	.16
160	14.78	+ 11.35	29.15	.00

TABLE XXVIII.

To show the influence of Size and Grouping on the Goodness of Fit (*P*).

Values of *P* which result when the groups are reduced to a total of 44 villages in each case and only 5 classes are used.

ROHTAK.		AMRITSAR.		MOZUFFARNAGAR.	
Group	Goodness of Fit (<i>P</i>)	Group	Goodness of Fit (<i>P</i>)	Group	Goodness of Fit (<i>P</i>)
200—300	.32	150—200	.16	500—750	.60
300—400	.34	250—300	.07	750—1100	.08
500—700	.46	200—400	.28	1100—1400	.21
400—1700	.75	400—1000	.24	1400—2200	.44
000—2950	.47	1050—1600	.15	Greater than 2000	.10
		Greater than 1600	.57		
Mean	.47	Mean	.25	Mean	.29*

* Two Rohtak and one Mozuffarnagar Groups contain four classes only, reduced to a total of 44, they give :

R. 700—900 .82 R. 950—1200 .40 M. 350—500 .87

TABLE XXIX.

Amritsar villages tested for recurrences in successive years of plague.

1. GROUPS USED.

Population limits	No. of villages in the group
1200—1400	46
1000—1200	67
800—1000	97
500—700	166
400—500	97

2. RESULTS ARRANGED IN GROUPS.

Group	1903 and 1904		1904 and 1905		1905 and 1906		1906 and 1907	
	No. affected in both years	Expected no. affected in both years	No. affected in both years	Expected no. affected in both years	No. affected in both years	Expected no. affected in both years	No. affected in both years	Expected no. affected in both years
1200—1400	25	23·65	27	27·83	17	14·78	10	12·93
1000—1200	26	25·03	33	30·85	25	24·52	23	22·21
800—1000	32	28·93	41	39·36	31	26·53	28	23·42
500—700	39	30·27	45	42·38	20	17·71	20	12·99
400—500	16	14·65	15	16·44	16	15·88	13	14·14
Goodness of Fit (<i>P</i>)	·54		·91		·84		·25	

3. TOTALS ARRANGED IN YEARS.

Years	No. affected in both years	Expected no. affected in both years	<i>P</i> = ·29
1903—4	138	122·53	
1904—5	161	156·86	
1905—6	109	99·42	
1906—7	94	85·69	

TABLE XXX.

Percentages of villages affected in any one year which did not report cases in the previous year.

Population limits	Infected in 1904 but not 1903 (percentage of all villages infected in 1904)	1905 but not 1904	1906 but not 1905	1907 but not 1906
1200—1400	18·8	32·5	0	71·4
1000—1200	30·8	37·7	9·7	52·1
800—1000	30·4	49·4	3·1	60·6
500—700	41·8	57·1	28·6	74·0
400—500	41·4	72·7	35·7	73·5

PART III. GENERAL SUMMARY OF CONCLUSIONS.

(1) While there is an appreciable number of villages in which the interval elapsing between the last death in one epidemic and the first death in the next following epidemic is so short that it is unnecessary to postulate a re-introduction of the disease, in the great majority the interval of freedom is so long that a re-importation of the infective agent is more likely to be the cause of the outbreak than recrudescence.

(2) The villages in which the infection has been, or may have been, carried over from one epidemic to the next are generally of large size and, with the exception of certain large towns, vary from year to year.

(3) A study of the later plague history of villages infected in the milder epidemics and of villages infected at the ends of epidemics confirms the conclusions stated in (1).

(4) A study of the distribution of infected villages in maps showing the position of affairs month by month suggests a spreading out of the infection from various centres, although, when the infection becomes widespread, this may be difficult to trace.

(5) Paying no attention to the respective sizes of the different villages, the numbers attacked in none, one, two, etc. epidemics do not form a random, or chance, distribution.

(6) There is a direct relation between the number of epidemics in which villages have been attacked and the average population of such villages. The average population of villages which have never been attacked is very small.

(7) The chief factor in the non-random distribution mentioned in (5) has been the great variation in size of village within each district. In Amritsar some other factor has also been very influential, in Mozuffarnagar and Rohtak no agency other than discrepancies in size can be definitely shown to exist.

(8) The incidence of plague in the Amritsar district is unlike that found in the other two districts.

(9) The statistical evidence does not point to the conclusion that the fact of a village having been infected in any one epidemic renders it more liable to be infected in the next following epidemic.

(10) Certain villages appear to possess an immunity distinct from the relative immunity conferred by low average population.

XXXVI AND XXXVII. OBSERVATIONS ON PLAGUE
IN BELGAUM AND POONA.

IN a former number of these reports (*Journal of Hygiene*, vol. VIII. p. 266) in an article dealing with the "Seasonal Prevalence of Plague in India," an endeavour was made to reconcile the facts of seasonal prevalence with the view that the transmission of the plague bacillus from rat to rat and from rat to man was effected through the agency of the rat flea. We demonstrated that plague could not exist, in epidemic form, in any of the six places that we selected for study, when the mean daily temperature was as high as 85° F. When this temperature is reached plague epidemics receive a check and rapidly decline. While this was so we pointed out that epidemics could and occasionally did decline and come to an end at times when the temperature appeared to be most suitable. It was clear that in such instances other factors come into play. In no places was this fact so well demonstrated as in Belgaum and Poona. It was for these reasons that we selected these two towns as the most suitable for further observations. We hoped to be able to throw some light on these other factors, and their relative importance. Climatic conditions appeared to be favourable to plague all the year round in Belgaum and for the greater part of the year in Poona.

It will be convenient here to recapitulate the propositions with which we closed the above quoted communication. We therein stated, "The rise of the rat epizootic and in consequence of the human epidemic depends upon:

(a) A suitable mean temperature somewhat below 85° F. and in general over 50° F.

(b) A sufficient number of susceptible rats.

(c) A sufficient number of rat fleas.

The fall of the rat epizootic and in consequence the epidemic, is determined by some or all of the following factors:

(a) A high mean temperature, 85° F. and above.

(b) A diminution in the total number of rats and an increase in the proportion of immune to susceptible animals.

(c) A diminution in the number of rat fleas."

How far these propositions hold good in the case of Poona and Belgaum, where the factor of a high mean temperature can be excluded, will become apparent from a perusal of the following pages. The Commission takes the opportunity of tendering their thanks to J. Carmichael, Esquire, I.C.S., Collector of Poona, and B. A. Brendon, Esquire, I.C.S., Collector of Belgaum, for their cordial assistance freely rendered at all times. The thanks of the Commission are also due to the Municipalities of Poona, Belgaum and Shahapur and the Cantonment authorities of Poona and Belgaum for their cooperation and interest in the work carried out in the districts under their control.

XXXVI. OBSERVATIONS ON PLAGUE IN BELGAUM, 1908—1909.

I. GENERAL DESCRIPTION OF BELGAUM DISTRICT.

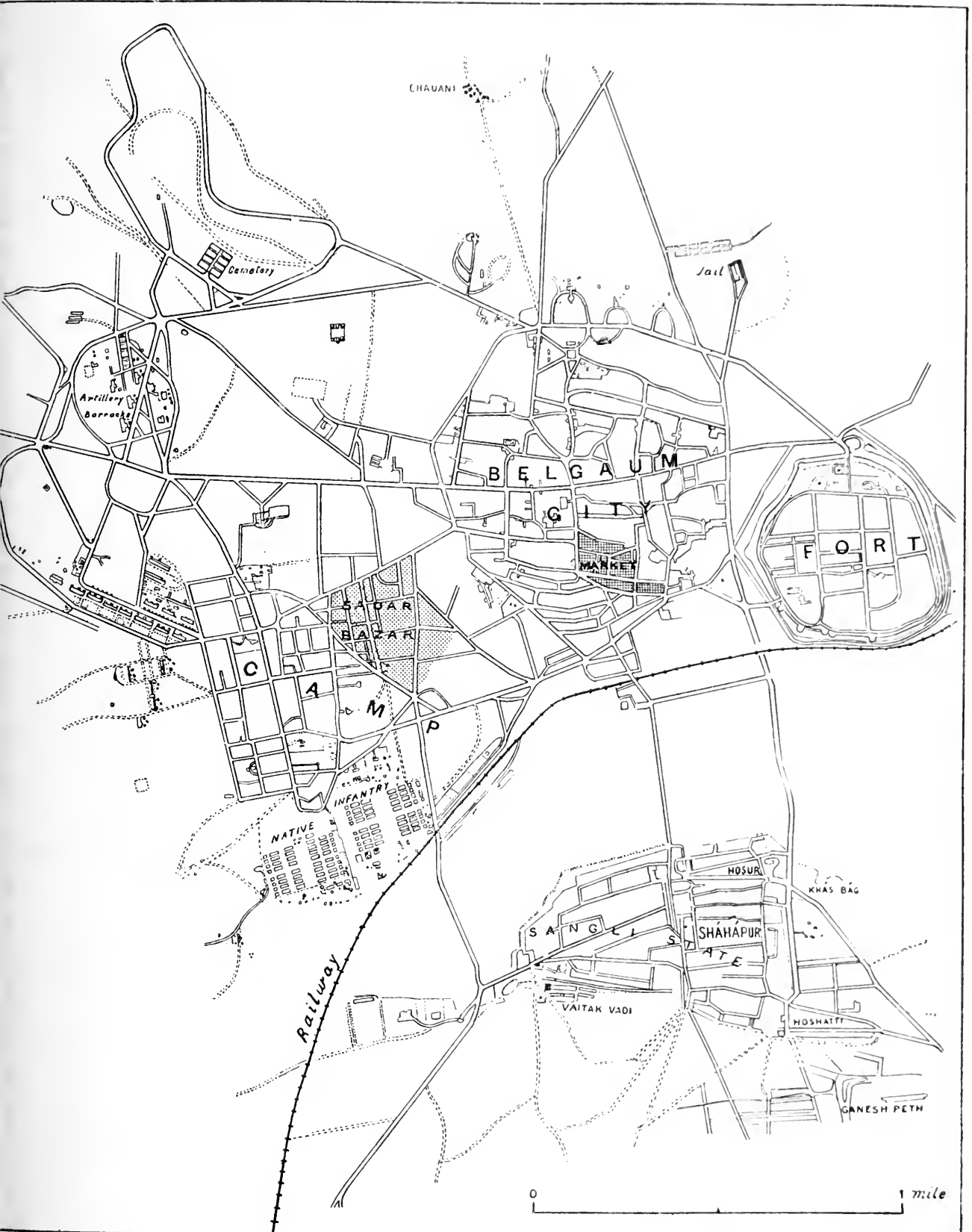
(a) *General description and geographical position.* Belgaum is a small town and military cantonment situated in the southern division of the Bombay Presidency, 75 miles inland from the western sea coast of India. It is situated $15^{\circ} 51' N.$ and $74^{\circ} 31' E.$ It lies on a plateau 2500 feet above the sea level on the northern slope of a water-course. The town is built on laterite which lies upon Deccan trap. The country round is fertile and well wooded.

Belgaum cantonment is a military station of some importance. It is the headquarters of a brigade in the Poona Division of the Southern Army. It has a garrison of four battalions of infantry and a battery of field artillery. The cantonment is divided into two portions, the Camp and the Fort. The Camp contains the lines of the four regiments and the battery and the officers' bungalows. The Sadar Bazaar is in the Camp on the side adjoining Belgaum city.

The Fort is roughly circular in outline with a diameter of 1000 yards; it is of considerable antiquity, being reputed to be about four hundred years old. It is surrounded by a deep moat. In the Fort are some twenty bungalows. With the exception of officers' servants it has no native population.

Belgaum city is a small town of considerable importance. It is the headquarters of the Belgaum District, a district covering 4649 square miles with a population of nearly a million people. The city lies between the two portions of the cantonment, viz. the Fort on the east and the Camp which extends along the western face of the town. It is roughly elliptical in outline and abuts on the Southern Mahratta Railway line which helps to form its southern boundary. The longest axis of the city north and south is about one mile, the shortest 1300 yards. South of the railway line, half a mile distant from Belgaum, is the small town of Shahapur. Shahapur belongs to the Sangli Native

MAP I



BELGAUM CITY AND ENVIRONS

N.B. The "Sadar Bazar" is marked in light hatching. The "Market" is marked in darker hatching

State. Hosur, Khasbag, Hoshatti and Vaitak-Vadi which geographically are portions of Shahapur are suburbs of and belong to Belgaum (vide map No. I). Juna Belgaum and Vadgaon belong to the Kurundwad Native State.

(b) *Climate.* Belgaum being only 75 miles from the sea and on the summit of the western Ghauts is exposed to fresh sea breezes that render its climate very equable. For descriptive purposes the year is best divided into :

(1) *The cold dry season* lasts from mid October to the end of February. The minimum temperature recorded in this season of the year is about 52° F. with a mean daily temperature about 70° F.

(2) *The hot dry season* follows the cold dry season and ceases with the advent of the monsoon early in June. The maximum temperature even in this hot season only occasionally reaches 100° F. and rarely exceeds this. The mean daily temperature ranges round 80° F. The nights are cool and pleasant. Heavy showers attended with the easterly winds, thunderstorms and hailstorms are of frequent occurrence in April and May.

(3) *The wet season* lasts from early June till about the middle of October. As would be expected from its position Belgaum derives full benefit from the south-western monsoon which is very constant in this part of India. The average rainfall is 50 inches most of which falls in the wet season. During 1908 the total rainfall was 47·91 inches distributed as follows: March ·09, April 1·20, May 1·57, June 5·91, July 23·69, August 10·76, September 3·74, October ·83 and November ·12. It will be noted that nearly half the total rainfall fell in July. The rainfall of August, September and October was below the average for these months, that of July in excess of the average. The hygrometric curve closely follows the rainfall curve. We shall have occasion to discuss hygrometric variations when describing their effect on the seasonal prevalence of the rat flea (vide Chart No. II).

(c) *Population.* The population of Belgaum city according to the census returns of 1901 was 26,237. This number included the suburbs of Belgaum, the population of which was returned as 3803. The population includes Mahrattahs, Brahmins, Mahomedans, Lingayats, Jains and Mahars in this order of numerical superiority. Mahratti is the language most commonly spoken. Canarese is a dialect used by the Lingayats, Jains and Mahars.

The population of Belgaum cantonment was returned as 10,641. This number included all troops and followers. The figure is now

somewhat too low. The only congested area in the cantonment and the only portion of it that we shall have occasion to consider in this report is the Sadar Bazaar Camp. The Sadar Bazaar covers an area of thirty-eight acres and has a population of three thousand five hundred¹.

Shahapur has a population of 9071, of similar constitution to that of Belgaum city.

(d) *Occupation, industries and trades, etc.* The population of Belgaum is for the greater part an agricultural one. Cotton weaving with hand looms is the chief industry both of Belgaum and Shahapur. A certain amount of silk spinning is also carried on. Copper and brass work gives occupation to a limited number of people. The most important articles of trade are salt, tobacco, dried fish, cocoanuts and coir which are imported by road from the coast, chiefly Vengurla. Grain of all kinds, tobacco, mollasses and sugar are brought in from the country round. There is a considerable trade in cattle.

There is a weekly market held on Saturdays, at which the townsfolk and villagers living within a radius of ten miles, or even more, obtain their supplies. A weekly market, an institution that is common to all towns of any size in the district, is probably of considerable importance as a factor in the dissemination of plague. To this point we shall have occasion to again refer. The market itself is situated near the centre of the town. Grain is here stored in large godowns. Each Saturday sees the market thronged with a vast concourse of people intent on disposing of their wares or laying in a supply of stores.

Most of these remarks about the inhabitants of Belgaum city apply equally to those of Shahapur. Here too there is a weekly Saturday market run on the same lines as, though on a smaller scale than, the Belgaum market.

(e) *Construction of houses.* The common type of house in Belgaum is well shown in the accompanying illustrations (Plates IX and X). With but few exceptions the houses are single-storied dwellings. Nearly all are built of mud. The outer walls are from one to two feet thick. Most of these houses are raised on plinths of varying height. These plinths are, like the houses, built of mud but the majority are faced with stone. The stones are plastered together with mud except in a few of the better class buildings. The floors are of mud or beaten down earth covered with a layer of cowdung. The roofs are of country tiles resting on several layers of interlacing bamboos, the whole being supported by rough wooden beams. Many of the houses

¹ Our own census July 1909.



Interior of a typical room in Belgium: the bags etc. contain grain.



Other end of same room: the large "kangi" and sacks contain grain.





Corner of Belgaum market, showing native shops.



A native house of the poorer class in Belgaum.



boast a verandah, but it frequently happens that the verandah, where it once existed, has been closed in by bamboo matting or other material to form an additional living room. Windows exist in a fair proportion of houses, but even when present they are often boarded in, or closed up with piles of stores or rubbish, so that it may be said that windows, functioning as such, are almost non-existent except in the houses of the fairly well to do. These houses of one, two, three or occasionally four rooms are nearly all dark dingy dwellings, the door being the sole source of air and of what little light succeeds in penetrating into their dim recesses. Chimneys there are none. The smoke escapes as best it may through the tiled roof. For all this many of the houses appear to be not badly ventilated, gaps between adjacent tiles of the roof forming efficient outlets for the vitiated air. Nearly every house contains its store of grain; the grain is stored in the living rooms, often in sacks, frequently in cylindrical receptacles made of bamboo matting. Both of these methods of storing grain are illustrated in the accompanying plates. In many houses there is a platform erected six or seven feet high, made of bamboos and supported on bamboo uprights, which serves as a convenient place for storing fodder, stores and rubbish. Not infrequently cattle share with their owners the shelter of these humble abodes. Overcrowding in some of these houses is appalling. The worst instance of this that we met with was in the case of a very poor house in Shahapur. It was a house of three fairly large rooms and a verandah. It was occupied at the time of our visit by twelve adults, three children, four buffaloes, six bullocks, a goat, a dog, a cat and three fowls¹. This is an extreme case but instances of a state of affairs nearly as bad are far from uncommon. There are of course houses of a vastly superior type to the picture we have drawn of a typical Belgaum house but they are comparatively few in number and with them at present we have no concern. The houses of the poorest classes again are merely single-roomed mud huts with a tiled roof. The inhabitants of these dwellings sleep on the floor. Charpoys (beds) so common in the Punjab even amongst the poor classes are very rarely seen in the houses of the poor and lower middle classes of Indians in this part of India.

The houses with the exception of a few boxes boast no furniture. Cooking is done in one of the living rooms of the house. Wood, or amongst the poorer classes cowdung cakes, is the fuel by means of which all cooking is carried out.

¹ On the abundance of animals see Appendix p. 478.

We have at least said enough to demonstrate how admirably suited a typical Belgaum house is to the requirements of the rat population. Shelter and food there are in abundance and usually freedom from molestation.

(f) *Sanitation and water supply, etc.* The sanitation of Belgaum city is in the hands of a Municipality. Shahapur has a Municipality of its own. The cantonment authorities are responsible for the sanitation of the Sadar Bazaar Camp.

Most of the houses in Belgaum city have latrines of their own. These are cleaned at night by municipal sweepers and the filth carted out to trenching grounds a mile or so from the city. A similar method of refuse disposal was till recently carried out in cantonments. Incinerators are now used to a certain extent.

The sanitary arrangements in Shahapur are more primitive. Here the poorer classes go out to the surrounding fields for purposes of nature. About half the houses of Shahapur have cesspits. No attempt is ever made to clean these and the cesspit is often in dangerous proximity to the well which serves as the source of the drinking water for the establishment.

The water supply is entirely from wells. Many houses have small wells of their own. In Belgaum city there are in addition several well-constructed large municipal wells.

Scavenging of the streets is done by sweepers employed by the Municipality. Along either side of most of the roads immediately in front of the dwellings runs an open drain, made of stones plastered together with mud varying in depth from 1 to 2 feet. This serves the purpose of carrying off storm water, as well as in too many cases forming the receptacle for street and house sweepings. As Indian country towns go, however, Belgaum is comparatively clean and well kept. There are parts of the town, however, that are very dirty. There is a sufficiency of open spaces in many parts of the town. In others the houses are sadly crowded together. In Belgaum city there are approximately 4650 houses which gives an average of a little over five people to a house.

(g) *Reference to former epidemics of plague.* Their great though decreasing severity and constant seasonal prevalence. A short account of the epidemics of plague that devastated Belgaum in the years 1897—1906 was given in a former number of these Reports (vide *Journal of Hygiene*, vol. VIII. pp. 270—277 and charts). It will be remembered that the seasonal prevalence was remarkably constant (see Table IV):

excluding the year in which plague was introduced (1897) all the epidemics, with the exception of one, commenced in July or August, reached their maximum severity about October, and declined in December and January. How severe some of these early epidemics of plague were, will be gathered from the fact that as many as 300 cases have been reported in a week at the height of the epidemic and this in a town with only a population of 26,000 people. The total number of deaths from plague in the Belgaum municipal area and cantonments since plague first started in 1897 is 12,108.

The earliest epidemics were the most severe; since when each epidemic, except that of 1901—02, was milder than its predecessor till 1906 when there was no epidemic at all. Plague reappeared in Belgaum in September 1907. This was a comparatively mild outbreak. There were 257 reported cases between September 1907 and May 1908 with 159 deaths distributed as follows:

September	6
October	23
November	65
December	60
January	36
February	39
March	18
April	10
May	0
	<u>257</u>

This small outbreak which cannot be compared in severity with its predecessors started later, reached its height a little later and declined very gradually, cases persisting into April.

When observations were started May 11th, 1908, Belgaum was apparently entirely free from plague.

(h) It will be convenient here to discuss shortly what prophylactic measures were being taken by the inhabitants against plague when the place came under observation. In Belgaum city a small amount of rat destruction had been carried out in times of epidemic but the scale on which this was done was entirely inadequate. In the cantonments a reward of one anna per rat was being offered. This measure was likewise quite ineffectual and resulted only in the destruction of about four or five rats a day. There was no attempt at any rat destruction in Shahapur. Inoculation was not practised except on a quite inappreciable

scale, the inhabitants being greatly prejudiced against the proceeding. On the appearance of plague a certain number of people vacated their dwellings and went to live outside the city, some in huts specially constructed for the purpose. A still larger number only left their houses when cases had actually occurred in them, *i.e.* too late to be of much practical value, and returned after a short interval. A very small amount of disinfection of infected houses was done by means of pesterine. The patients were, except in the case of the very poor, treated in their own homes.

II. SCOPE OF THE ENQUIRY.

In the general introduction the chief reasons have been given why Belgaum was selected as a place about which it was thought desirable to try and obtain information which might help to throw light upon the seasonal prevalence of plague in that district. We stated here that, in the light of our previous experience, climatic conditions in Belgaum appeared at no time of the year to be unfavourable to plague.

The problems that confronted us at the outset of the inquiry were:

(1) What factors especially favourable to plague exist in July and August which are capable of explaining the constant appearance of plague at this season of the year.

(2) What are the factors existing in December, January and February which render this period of the year unsuitable to plague, thus prompting the decline of epidemic.

(3) What happens to plague in the off season. Does it persist in acute form amongst rats, as in Bombay, or in a chronic form, or does it entirely disappear, each epidemic originating with a fresh importation of infection.

We hoped that our observations might incidentally throw additional light on the habits and life history, distribution and prevalence of rats and fleas. We also hoped to learn more about the interesting condition described by us in a previous report as chronic plague of rats.

Description of the methods which were adopted in Belgaum for studying the epizootic and epidemic and the habits of rats and fleas.

Our first task was obviously to make a study of the rodent population of the town and to this end arrangements were made for systematic trapping of the place. Rat examination was commenced on

12th May 1908. It was our first intention to limit our observations to Belgaum town but a little experience soon showed that the near propinquity of the Sadar Bazaar Cantonment and Shahapur rendered their inclusion in the field of our work desirable and necessary.

Work was commenced in Shahapur on the 19th June and in the Sadar Bazaar on the 4th July 1908. An average of 520 traps were set daily, about 80 in cantonments, 80 in Shahapur and 360 in the city (Belgaum proper).

The town of Belgaum is for municipal purposes divided up into twelve wards. Work went on simultaneously in all these wards and endeavours were made to trap the whole town evenly. We thus hoped that each day's catch would represent a fair sample of the rats of the town. The traps were in the charge of coolies who worked under the supervision of inspectors. Each cooly had charge of from 20 to 30 traps.

Account was kept (1) of the number of traps set daily; (2) the number of houses trapped and their addresses; (3) the number of rats caught.

Traps cleaned and rebaited were taken round and distributed each afternoon. The following morning the houses in which traps had been left were revisited and any rats caught collected. When a trap was found to contain rats it was put in a canvas bag, tied up and a label affixed. The label which was filled in by the inspector contained the following information: (1) serial number, (2) the address where the rat had been caught and (3) the number of rats in the trap. The rats thus caught were then despatched to the laboratory for examination. Endeavours were also made to obtain the corpses of any rats that might have been found dead (especially in the plague season) but our efforts in this direction met with very little success. The townspeople did very little to second our efforts, and though occasionally we received information that rats were dying in certain houses or in certain streets it was only on very rare occasions that the corpses of such rats were handed over to members of our rat-catching staff. We had to content ourselves, therefore, with studying the progress of the epizootic by examination of live rats, the number of dead rats obtained being very small indeed. For some reason or other the populace were even averse to confess that a rat mortality was taking place in their houses: on several occasions we found that this was the reason, however, that caused householders to suddenly vacate their houses. Undoubtedly, too, rats may be dying in a house without the inhabitants being aware

of the fact. Rats not infrequently die in their burrows, or under cover of boxes or sacks, or amongst rubbish or even in the roofs of the houses. In one case of plague that we were investigating an intelligent householder volunteered the suggestive information that four or five days prior to the date on which the patient was taken ill, he had noticed maggots falling from the roof of one of the living rooms. He had not seen any dead rats. On the whole people were not averse to taking traps into their houses. There are exceptions, however, and no amount of persuasion on our part could get round their prejudices. This objection to taking in traps was not wholly confined to the Jain community¹ though as was to be expected it was most marked amongst them. It is suggestive that the two roads where we had most trouble in this direction (viz. in Hosur) were more heavily infected with plague than any other portion of the town. The adjacent lanes in Shahapur which were being fairly constantly trapped at the time when Hosur was affected were comparatively free from plague.

On arrival at the laboratory the rats were submitted to examination much in the same way as in Bombay (vide *Journal of Hygiene*, vol. VII. p. 738). The trap was removed from the canvas bag, and trap and bag were at once placed in a tin box, provided with a well-fitting lid, and a removable tray covered with white american cloth. The trap (containing rats) and the bag were then freely sprinkled with chloroform and the box closed. After some minutes had elapsed the box was opened, the canvas bag vigorously shaken over the open box to detach any fleas that may have adhered to the bag, and then the tray and the trap which was resting on it were together removed and taken to the flea counting table. Here the fleas found resting on the tray were collected and counted and each rat examined separately for fleas. Banging the rat vigorously on the table for a minute or so is usually sufficient to dislodge any fleas that remain on the rat, provided that sufficient chloroform has been used. Usually it was found that about one quarter of the total number of fleas was found resting on the tray, the remainder adhering to the rats. The flea counting table was covered with white cloth. Having thus made as accurate a count as possible of all the fleas obtainable from the rats in any one trap the number of fleas was entered on to the trap ticket referred to above.

Another ticket was then made out for each rat. On this was written the serial number of the rat and the number of the trap ticket. The

¹ The Jains have strong religious scruples about taking the life of any animal: even parasitic insects such as bugs are carefully preserved and occasionally deliberately fed.

rat was then weighed, pinned out on a board and dissected in the manner that was described in detail in the account of the work in Bombay. All the information thus obtained was entered on the rat ticket, viz. species, weight, sex, if female whether pregnant or not, if pregnant the number of foetuses, whether healthy or not. If there was anything of interest, or if the rat appeared to be unhealthy, full notes of the condition and pathological changes were made on the back of the card. At the end of a day's work all this information recorded on the trap and rat tickets was entered in a special register.

Early in the work a spleen smear of every rat was made and stained with carbol thionine and examined microscopically. This practice as a routine was afterwards abandoned though it was adhered to in all doubtful cases. The results obtained were not commensurate with the amount of labour involved. The previous experience of the Commission was again confirmed, viz. that in the diagnosis of rat plague macroscopic appearances are, to a trained observer, of greater import than microscopic examination. The microscope was used merely as a confirmatory test in any case of doubt. When there was any further doubt as to the diagnosis cultural and animal tests were also resorted to.

Plague cases in Belgaum are reported to the Municipality. For these reports the Municipality rely on medical practitioners and an inspector who was employed by the Municipality for the purpose of discovering and reporting plague cases.

The Commission received daily notice from the municipal authorities of cases that had been reported to them. In addition they often received early news of cases from members of the rat-catching staff who were constantly employed in every part of the town. In a similar way news of plague cases in Shahapur was received from the Secretary of the Shahapur Municipality. Cases occurring in Sadar Bazaar Cantonment were reported by a Hospital Assistant on special plague duty to the cantonment magistrate who was kind enough to forward such reports to the member of the Commission in charge of the Belgaum work. Many cases, more especially those occurring early in, and on the decline of, the epidemic were verified by the Commission. A few mild cases undoubtedly escaped being reported and in a few instances cases reported as plague had been wrongly diagnosed as plague; but on the whole the information collected as described was accurate enough. From a careful study of deaths from all causes contained in the death registers we have arrived at the conclusion that very few deaths from plague escaped notification.

III. RATS AND FLEAS.

Rats.

The following species were met with in Belgaum :

Mus rattus

Bandicota indica (*N. bandicota*)

Mus musculus

Gunomys varius (*Nesokia bengalensis*)

as well as Musk Rats (*Crocidura coerulea*).

In the period during which the Belgaum observations were being carried out, viz. from 12th May 1908 to June 30th 1909, 39,460 *Mus rattus* were trapped. Of this number 38,957 were submitted to examination in the daily routine in the manner that has been described above. The remaining 503 were kept in stock for experimental purposes.

The other species were in comparison numerically insignificant. They were as follows:—1180 mice, 503 musk rats, 71 bandicoots and 3 *Gunomys varius*. Musk rats are very much more numerous than would appear from the above figures. Many of them were liberated when caught in deference to the sentiments of the Hindu population who hold this animal in high esteem. Its immunity to plague renders it harmless from the plague standpoint. A plague infected musk rat was never found by us in the Belgaum observations.

Mice too are comparatively more numerous than the figures indicate. Our traps were not suitable for mice.

Mus rattus.

This is the common species of house rat in Belgaum as in most parts of India. The type resembles closely that found in the Punjab, Bombay and Poona. It is perhaps a little larger than the Bombay variety and certainly much larger than the Poona *Mus rattus*. The average weight of an adult *Mus rattus* was between 140 and 150 grammes. Three specimens weighing 250 grms. were taken. It is essentially a house rat. It burrows frequently, but more commonly it lives and breeds in the house amongst heaps of rubbish, behind boxes or in the roof. Burrows of *Mus rattus*, however, have been seen in many houses. As will have been gathered from the description given

a typical Belgaum house, these dwellings leave little to be desired from the rat's point of view.

Breeding. The *rattus* of Belgaum breeds all the year round but to much greater extent during the first half of the year than the latter half. Approximately the breeding season of *Mus rattus* in Belgaum corresponds with the off plague season. Daily observations were made of the total number of *Mus rattus* examined, the total number of young rats (70 grms. and under) and the total number of pregnant females. The weekly figures are given in the annexed table (Table I). The average number of foetuses in 4841 pregnant *Mus rattus* was 5.4.

Degree of rat infestation. It is difficult to form an adequate idea of the degree of rat infestation of any given place. There are a few facts in this connection, in the case of Belgaum, however, that are worth consideration. During the period of our observations the number of *Mus rattus* caught was slightly in excess of the human population. When allowance is made for houses where the inhabitants refused to take traps, approximately six rats were caught for every house trapped. When trapping operations were started the number of rats taken per 100 traps set was about 32. When the observations ceased this figure had fallen to 16 (see Chart III and Table I). We may assume, therefore, that we had, with the assistance of the plague epizootic, very considerably reduced the rat population. We acknowledge that this gives us little or no idea of the exact rat population but it suffices to demonstrate to what a huge extent the houses were infested with rats. It will be noticed on referring to Table I, that the percentage of young rats was very much higher in May and June 1909 than it was in the corresponding months of the previous year. This indication of more intensive breeding was perhaps compensatory to the attacks on the rat population made by plague and our trapping operations. (The plague in Belgaum in 1908—09 was a good deal more severe than it was in 1907—08.) In spite of our continued trapping the rat population was apparently on the increase when our observations came to a close (see Table I).

*Immunity of *Mus rattus* to plague.* Our main object as we have already mentioned in undertaking observations in Belgaum was to endeavour to throw more light on the subject of seasonal prevalence of plague. With this object in view an endeavour was made to determine whether or not the immunity of the Belgaum *rattus* to plague underwent seasonal variations. Our original idea was to inoculate some 50 *Mus rattus* with plague each week and observe what percentage died of

plague. The difficulties that we encountered were numerous. Among these we may mention the great difficulty we experienced in keeping wild rats alive and well in captivity. We found that when the rat cages became damp, especially in the wet weather, many rats died from unknown causes, so that we had considerable difficulty in determining when a rat actually died of plague. Some rats for example were found dead on the second day after inoculation presenting no symptoms of plague except numerous plague bacilli in the lymphatic gland nearest the site of inoculation. Should such a rat be recorded as having died of plague or would it be more correct to class it with many others which had obviously died from causes associated with the unnatural surroundings in which they were kept? Another important difficulty arose from the impossibility of maintaining throughout a long period a constant test dose of plague bacilli. We started by using the cutaneous method of inoculation. A spleen or liver of a rat or guinea-pig dead of plague was rubbed into the shaven surface of the rat. Using this method we found that very few rats died of plague. Batches of twenty were done without a single plague death. We then tried injecting a high dilution of an emulsion of infected rat spleen in normal salt solution, selecting only those spleens which showed numerous plague bacilli on microscopic examination of smears. We found it impossible by this method to get a uniform dose week by week and our results were most irregular. Altogether we inoculated 264 rats and then abandoned the experiment. The one lesson we learned from the experiment was that the *rattus* of Belgaum appeared to enjoy a very high degree of immunity.

With the object of comparing this immunity with that of the *rattus* of Bombay and Poona, rats were sent from Belgaum and Poona and put into the godowns at Parel along with Bombay rats. Into each godown were put equal numbers of Poona, Bombay and Belgaum rats. Each rat was in a separate cage on the floor of the godown. Into each godown very numerous plague infected fleas were introduced. In all, experiments were done on 270 rats with the following results:

Of 90 Poona rats	24	died of plague.
Of 90 Bombay „	16	„ „ „
Of 90 Belgaum „	8	„ „ „

From this experiment it would appear that the Belgaum¹ rat is

¹ It may be worth noting in this connection that in proportion to their populations Belgaum (population 26,000, plague deaths 12,000) has suffered much more severely from human plague than Bombay (about 900,000, plague deaths 167,000).

twice as immune as the Bombay rat and three times as immune as the Poona rat to plague, but it is obvious that the figures are not large enough to warrant any reliable deductions being made from them.

Bandicota indica.

During the year 71 bandicoots were captured. This number does not give an adequate idea of the relative frequency of this rodent but as compared with *Mus rattus* it is certainly rare. The traps that were generally used by us were not nearly large enough to capture bandicoots and the specimens we obtained were caught for us by the townsfolk in large wooden country traps. A reward was offered for every bandicoot brought in.

The bandicoot of Belgaum is a large coarse-furred rat. It is dark grey in colour. It has soft thick fur from which protrude long bristles. Its tail is about equal in length to the length of the body and head combined. It is far bigger than any other species of rat that is met with. We have had several specimens weighing over 1000 grms. A full grown specimen cannot be mistaken for anything else. A very young one might be mistaken on cursory examination for *Gunomys varius*. When our observations commenced the bandicoot was found only in three or four streets on the outskirts of the town. In these streets it was not uncommon. In April, May and June 1909, however, we obtained specimens from various parts of the town. In its habits it is a house rat. Its burrows are usually to be found in the mud floors of a native house, occasionally in the walls. In spite of its enormous size the people do not appear to resent the presence of bandicoots in their houses. One man, whose house harboured a colony of these animals, refused to let us trap them for said he "if we have bandicoots we shall not get plague," a popular but dangerous fallacy as we will presently show. The burrows of the bandicoot are occasionally of gigantic size, far bigger than would appear to be necessary from the size of the animal; they resemble rabbit warrens, and often literally undermine the floors of native houses. The burrowing capacity of the animal is truly remarkable. The amount of damage that it is capable of doing in a single night would appear incredible to any one who had not ocular demonstration of its burrowing abilities. The bandicoot that we have met with in Belgaum is remarkably susceptible to plague—very much more so than the *rattus* of Belgaum which as we have seen possesses a comparatively very high degree of immunity. A full grown bandicoot

on one occasion succumbed in three days to a dose of plague that failed to kill a guinea-pig of considerably less weight. For this reason, and the fact that the animal lives and thrives well in captivity, we have frequently employed bandicoots for laboratory experiments in the place of guinea-pigs to determine the identity of plague-like organisms. In Belgaum as in many other places in the Bombay Presidency there is a well authenticated report that before plague came the bandicoot was the rat most in evidence in every quarter of the town. There was scarcely a house without its colony of bandicoots. With the advent of plague the bandicoot disappeared, or migrated as the report usually has it. Its disappearance can be readily credited, though we have obtained no reliable evidence of migration. Its remarkable susceptibility to plague is quite sufficient to account for its disappearance from Belgaum city. Further report says that until the present year the bandicoot has been conspicuously absent. It is only just beginning to return. This is credible. It has not yet "returned" to Shahapur where it was once equally common; we only obtained two specimens from the cantonment and these were both found dead of plague. The comparative mildness of the last few epizootics of plague would account for the reappearance of the bandicoot.

In this connection it is interesting to note that nearly all bandicoots caught in Belgaum were caught in the off plague season. None were brought in between the end of August and January in spite of a reward of eight annas offered for each one brought in alive. The disappearance of the bandicoot on the advent of plague was also noted in Hindulgar, a small village three miles from Belgaum.

Gunomys varius.

We have only met with three specimens of this rat. All three were caught in a field at some distance from human habitation. It does not occur as a house rat in Belgaum.

Fleas.

The only species of rat flea that we have met with in Belgaum is *Loemopsylla cheopis*. Two specimens of a flea very similar to *Ceratophyllus fasciatus* were found, together with four *L. cheopis*, on a squirrel dead of plague. The former species was never seen on a rat in Belgaum.

The flea infestation of *Mus rattus* in Belgaum is very large, and greater than has been observed in other parts of India where similar observations have been made. There is, too, a very noteworthy seasonal prevalence of fleas. A reference to Charts I, II and III and to Table I will make these points clear. The average number of fleas per *Mus rattus* varied from 3·6 in May to 18·6 in October. During 13½ months 380,678 fleas were counted on 36,890 *Mus rattus* which gives an average for the whole period of 10·3 fleas per rat. The average for *rattus* in Bombay was 3·8, in Dhand 9·1, in Kasel 6·9, in Poona 5·9, and for *decumanus* 8·4 in Bombay.

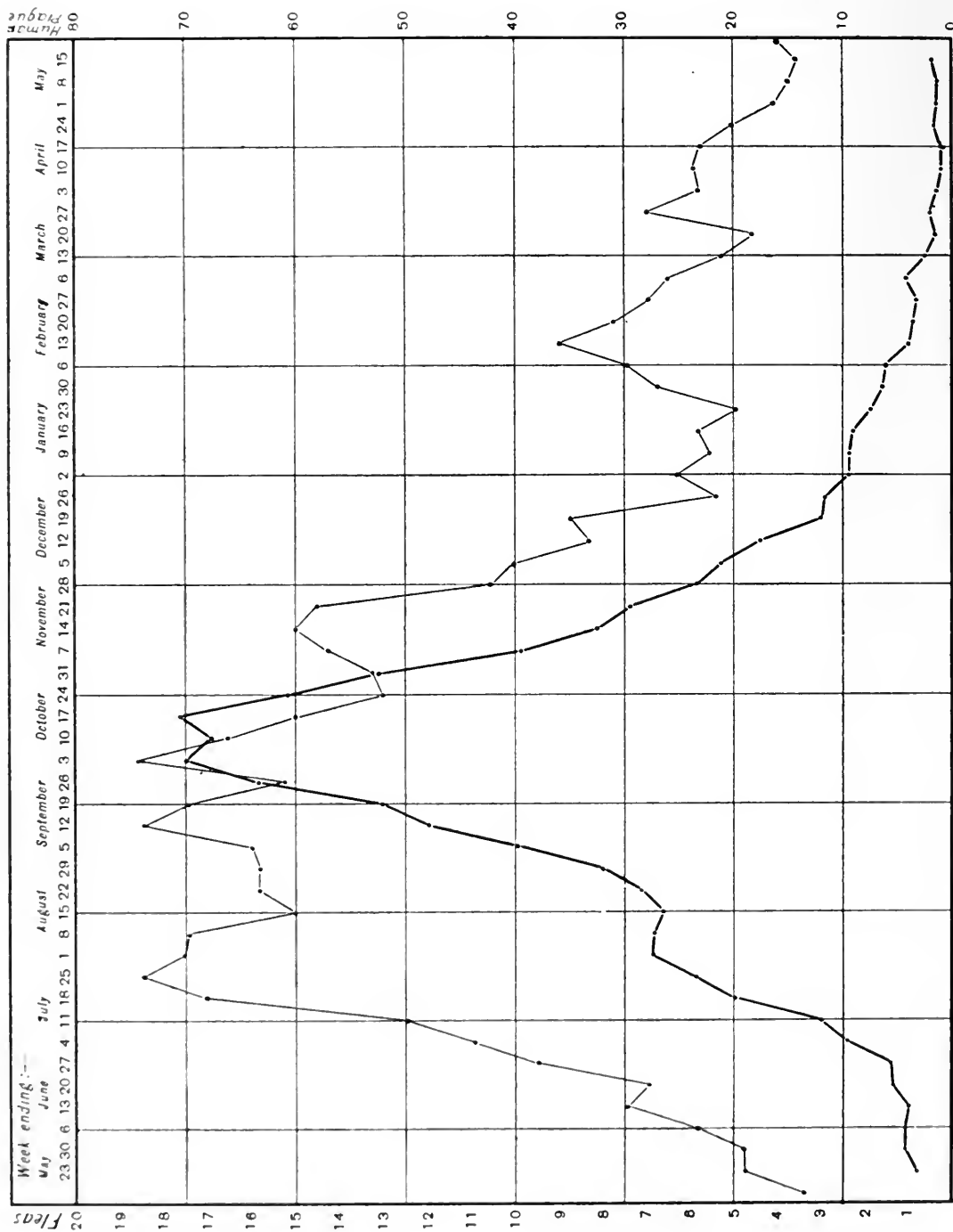
Chart II demonstrates the correlation which appears to exist between flea prevalence and the amount of moisture in the atmosphere. That this is not an absolutely constant correlation is evident from the fact that a similar state of affairs does not hold good for Bombay. We believe it to be, however, a true correlation within limits of temperature,—in other words, provided the mean daily temperature is above 60° and below 80° F. flea prevalence varies directly with the hygrometric conditions of the atmosphere. Speaking generally it would appear from the curve depicted in Chart II that a rise or fall in humidity is followed after an interval of three or four weeks with a corresponding rise or fall in the flea count.

We have not been able to find in Belgaum any host of *Loemopsylla cheopis* other than rats, with the exception of the squirrel found dead of plague referred to above. Goats, dogs, mongooses, civet cats, tame monkeys, turkeys, chickens, ducks, pigeons and bats have been examined for *L. cheopis* without yielding a single specimen.

IV. PLAGUE EPIDEMIC AND EPIZOOTIC 1908—1909.

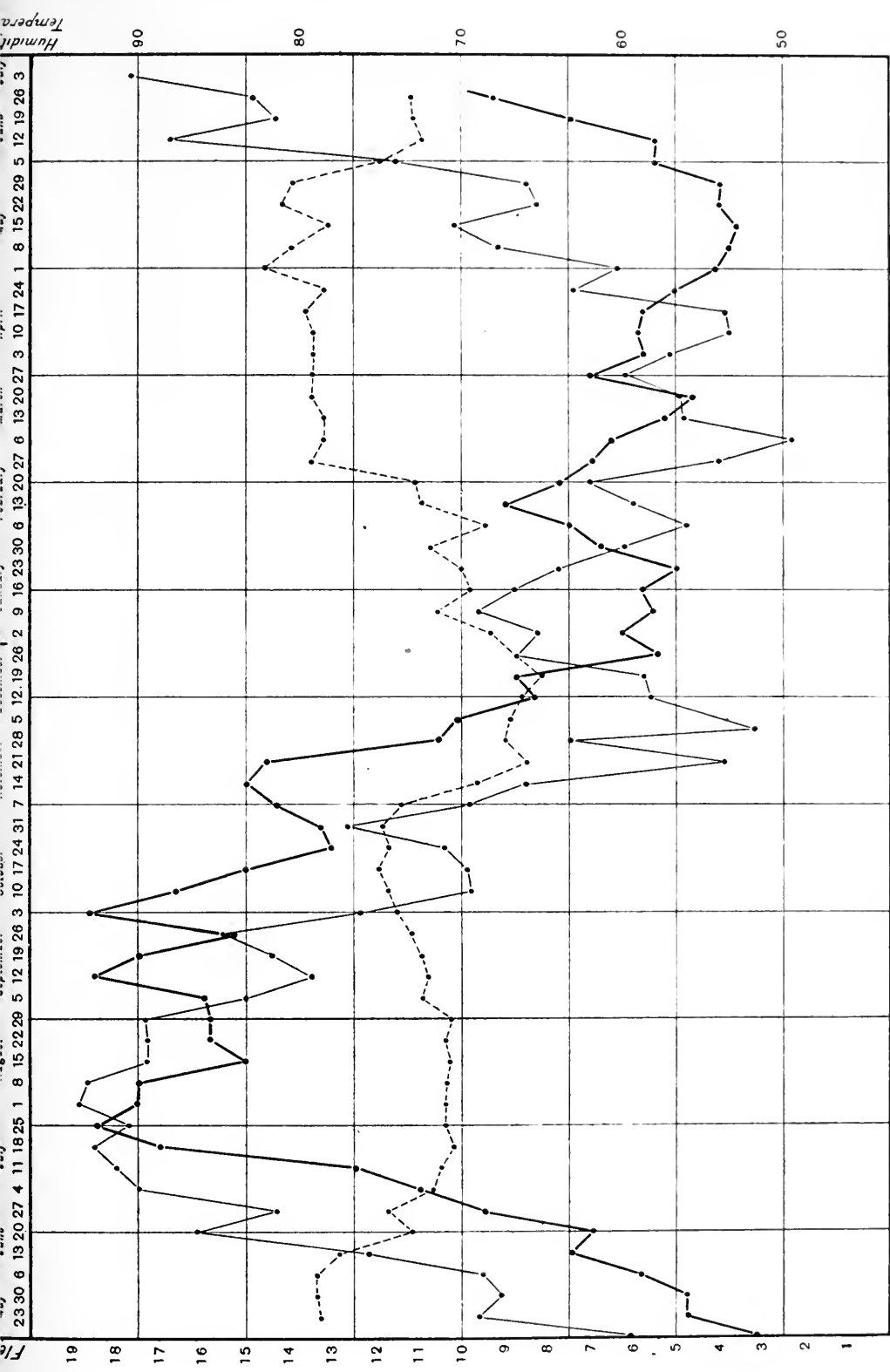
Observations were started in Belgaum on 12th May 1908, that is to say in the middle of the off plague season. At that time careful enquiries elicited the fact that human plague was entirely absent from Belgaum. The last case that had been reported was during the first half of April. May was entirely free from plague but on June 5th a case (Ranubai Laxuman, 4115 Jalgar Gulli) occurred in Belgaum City. The case was seen by us and verified as typical bubonic plague. There was no history of dead rats in the house: in fact the inmates denied the presence of any rats as “several cats frequented the house.” Rats must however have been there for a guinea-pig was left for one night in the house, and yielded five *L. cheopis*; none of these contained plague-

CHART I



BELGAUM

— Average number of fleas per Mus Rattus: weekly figures: observations



BELGAUM

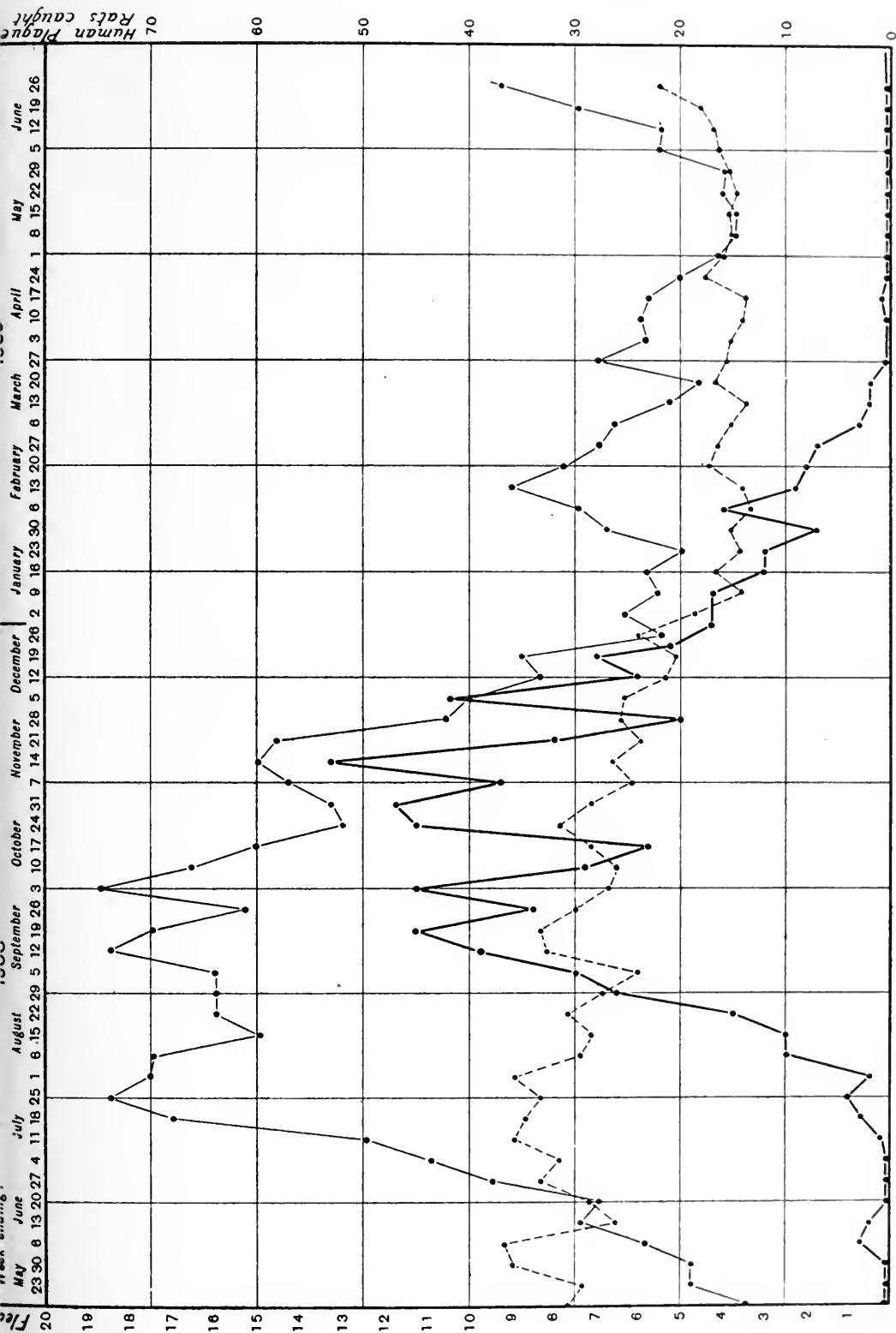
- Humidity
- Average number of fleas per Mus rattus
- Mean temperature. (Fahrenheit)

like bacilli and the guinea-pig remained well. The woman died on the 6th June. Two days later a second case occurred in a neighbouring house (Laxumbai Punapa aged 25, 4126 Jalgar Gulli). The patient was the widow of a man who was stated to have died of plague in the latter half of April, her only child dying a day or two after her husband. This case also ended fatally. The diagnosis of this case was confirmed bacteriologically. A virulent culture of *Bacillus pestis* was obtained from the bubo. Here again we could get no evidence of any mortality amongst the rats. On the 10th June the son (Parashuram Laxuman) of case No. I was attacked with plague in a house 4110 Jalgar Gulli into which the people had moved on the death of case I. He died on 13th June. Rats were being caught in adjacent houses at the time these cases were occurring but we could get no evidence of acute rat plague. These three cases formed an isolated outbreak. They were the only cases that occurred in Belgaum City in the month of June. We believe the infection was indigenous: there was no evidence to the contrary. A guinea-pig subsequently lived for a week in 4115 Jalgar Gulli, the home of the first case. It remained well.

In the meantime there had been two cases of plague in the Sadar Bazaar Camp. A mother and her son were both taken ill with plague on 2nd June with symptoms typical of bubonic plague. They both recovered.

These early cases are of importance as they indicate that indigenous plague occurs in Belgaum in the off plague season without assuming epidemic proportions. We shall endeavour to advance a satisfactory explanation of this subsequently.

Nothing further of any interest happened till the 11th July 1908. On this day a rat caught alive and apparently well in 27 Kondappa Street Camp revealed on post-mortem examination macroscopic appearances very suggestive of plague, and was proved to have been plague infected by culture and animal experiment. It subsequently transpired that on the same day a girl aged six years was attacked with plague in a neighbouring house (32 Kondappa Street). This patient died on the 14th July. A guinea-pig left to run in the patient's house picked up seven rat fleas, two of which had plague-like bacilli in their stomach contents. On the 18th July a second plague rat was caught close by in 33 Market Street. A guinea-pig was sent to live in this house in a cage for a week. On its removal only four rat fleas were found on it but the guinea-pig died of typical acute plague on the day following its return to the laboratory. From this time on plague gradually spread and



BELGAUM

- Human Plague Cases (Belgaum City, Suburbs, Cantonment and Shahapur)
- Number of fleas per *Mus rattus*
- Number of rats per 100 traps set

assumed epidemic proportions. During July six rats caught alive in the Sadar Bazaar were plague infected and on August 1st three rats dead of plague were found in the Police Lines Sadar Bazaar. In Belgaum City seven cases of plague occurred in July. All these cases occurred on the side of the town that adjoins the Sadar Bazaar Camp (see map No. II) and in four cases at any rate the infection was traced with more or less certainty to the latter place. Thus started the 1908—09 epidemic of plague in Belgaum.

It is difficult to speak with any certainty as to the source of the infection that started the epidemic. The following facts are, however, of interesting significance. On July 14th a boy Parasharam Krishna was admitted into the plague hospital suffering from undoubted plague. This was the first case of the epidemic that had occurred in the city. He was brought to hospital from a house in which he had only been living two days. Previously to this he had been living in the Sadar Bazaar where his father had died of what was very possibly plague on the 12th July; his death had, however, been ascribed to other causes. There was a history that this family had recently come from a village some twelve miles away where there was plague. It was certainly from the neighbourhood of their house in the Sadar Bazaar that the epidemic of 1908—09 started.

Comparing the different behaviour of the little outbreak that occurred in June and the one that followed some five weeks later, we note that, whereas the former was self limited the latter rapidly assumed epidemic proportions. A reference to Chart No. I will show that early in June the rat flea prevalence was low and amounted to only five fleas per rat¹. In the first half of July the flea count gave fifteen fleas per *Mus rattus*.

Meanwhile plague had made its appearance in Shahapur. Two rats caught alive by us on the 14th July were found to be plague infected. Plague was "declared" in Shahapur on the 2nd August. Four or five cases were, however, seen by us during the last week of July. As was stated above our Shahapur observations only started on the 24th June 1908. At that time Shahapur was free from plague. A fortnight previously, however, a case had occurred in Shahapur. It was diagnosed as plague by an experienced practitioner well versed in the symptoms of this disease and we have little doubt as to the correctness of the diagnosis. Two cases occurred in Hosur during the last week of July.

¹ Five fleas per rat however is enough to spread plague in Bombay.

The subsequent history of the epidemic will best be appreciated by a reference to the maps Nos. II—XVII and Chart III. It will be seen how the epidemic started in the Sadar Bazaar Cantonment and gradually spread from there until it spread all over the city. A second early focus of infection was in Shahapur and a third in Hosur (vide maps Nos. III and IV). Hosur consists of two streets on the north side of Shahapur. When plague appeared in Hosur it was not being trapped nor had we previously caught any rats there. Directly plague broke out we commenced to trap the place. The commencement of our work in Hosur, however, being synchronous with a severe outbreak of plague, colour was lent to a rumour that had been started that we were spreading infection with our traps. People with one accord refused to take traps; and the inhabitants of Hosur suffered much more severely from plague than any other part of Belgaum or Shahapur. A glance at maps Nos. IV and V will show how free the streets of Shahapur neighbouring on Hosur kept from plague whilst Hosur itself was badly infected. These neighbouring streets are comparable in every way with the streets of Hosur and in very close proximity thereto. The one point of difference was that Shahapur was being fairly well trapped at the time whilst Hosur was wholly neglected.

The second point of interest that is brought out by the maps is the different behaviour of plague in the Sadar Bazaar Camp and Belgaum City. Plague broke out first in the Sadar Bazaar, reached its height in the first half of September and had completely disappeared by the middle of November. In the city the epidemic started later, reached its height in November and gradually declined, cases persisting until the latter half of March.

The two places lie side by side and free communication exists between them. On the whole the number of fleas per *Mus rattus* worked out the same for both places. The explanation of the difference between the two epidemics appeared to be due to a difference in the rat population, whereas in the Sadar Bazaar in the beginning of July we were able to catch upwards of 40 rats per 100 traps set, in the city we could only catch 30. Two causes apparently contributed to the higher rat infestation of the Sadar Bazaar:—

(1) There was practically no plague in the Sadar Bazaar, during the season of 1907—1908: only five deaths were returned, whereas in the city there were 257 reported cases with 159 deaths.

(2) Our trapping operations began in the city in the middle of May; in the Sadar Bazaar not till the beginning of July.

Although the rat population was higher in the Sadar Bazaar in the beginning of July it very rapidly declined so that by the end of September we were able to take only 15 rats per 100 traps set. The decline in the rat population of the city was much more gradual and it was not until the middle of January that our catches sank to the figure of 15 rats per 100 traps. Two causes were contributory to the more rapid decline of the rat population in the Sadar Bazaar :—

(1) The greater severity of the epidemic and presumably of the epizootic in the Sadar Bazaar than in the city. In the Sadar Bazaar with a population of 3500 there were 112 cases, *i.e.* 3·2% of the population were attacked. In the city with a population of 23,000 there were 405 cases, *i.e.* only 1·7% of the population were attacked.

(2) Trapping was carried out somewhat more energetically in the Sadar Bazaar than in the city. This is shown by the fact that in the months July to October 2332 rats were caught in the Sadar Bazaar—a number equivalent to $\frac{2}{3}$ of the human population—whereas in the city 9672 were trapped, considerably less than half the human population. The number of traps used in the Sadar Bazaar per 1000 human population was more than double the number used in the city. (From July to October 34,494 traps were set in the city and 9347 in the Sadar Bazaar.)

Which of these two factors was the important one in bringing the Sadar Bazaar epidemic to a close it is difficult to determine. That the severity of an epidemic is a most important factor in curtailing its duration is illustrated by Hosur. A reference to the maps will show how much more severe the epidemic was here than it was in Shahapur but of much less duration. As we have stated Hosur was untrapped for the most part.

During the epidemic there were 783 cases of plague with 516 deaths which give a case mortality of 65·9%.

These cases were distributed as follows :—

Belgaum City and suburbs 507 cases, 331 deaths, case mortality 65·2%.

Sadar Bazaar 114 cases, 80 deaths, case mortality 70·1%.

Shahapur 162 cases, 105 deaths, case mortality 64·8%.

As has been stated Hosur was much the worst infected portion of the area under observation. Math Gulli and Basawant Gulli Hosur have a human population of 649 (our own census June 1909). In these two streets alone there were 71 cases and 46 deaths. This is equivalent to saying that if the whole of Belgaum, Shahapur and the Sadar Bazaar had been as badly infected as Hosur there would have been an

epidemic in 1908—09 of 4230 cases instead of 782. We should hardly be justified in assuming that all this difference was due to the fact that fairly energetic rat destruction was going on in all parts of the area under observation except in Hosur, but the assumption is warranted that had it not been for the rat destruction that was going on Belgaum would have passed through a much more severe epidemic than it did.

By the beginning of April Belgaum was again quite free from plague.

Additional evidence to support the view that climatic conditions *per se* in the Belgaum district are never very unfavourable to plague was furnished by an interesting little outbreak of plague that occurred in the village of Hindulgar, which is situated three miles west of Belgaum. Here plague broke out in the middle of April, *i.e.* in the middle of the off plague season. There were in all eight or nine cases, one of which was seen by a member of the Commission on April 21st and verified as plague. There was a history of rat mortality. The epidemic never got a firm hold of the village. Several rats were trapped and examined. The flea prevalence was the same at the time as it was in Belgaum, *i.e.* about five fleas per rat.

The Epizootic.

During the year 130¹ rats were found to be suffering from acute plague. Of this number 110 were brought in alive and 20 dead. Of the latter several were caught alive and died in the trap before arrival at the laboratory. This number of acutely plague infected rats when compared with the number of human plague cases is very small. As has been previously mentioned however our attempts at obtaining the corpses of rats found dead were, for reasons detailed above, unsuccessful. This sufficiently explains why more acute plague infected rats were not found. In addition to the above, a large number of rats in various stages of recovery from plague were met with (see above p. 347). The time and place correlation between human plague cases and acute and resolving plague in rats is shown in the accompanying maps, but our information about the epizootic is obviously very incomplete.

Map No. VI shows that three rats suffering from acute plague were caught in the Fort at a time when plague was absent from the city but

¹ Of the 130 rats which were found suffering from acute plague 18 had no discoverable buboes. Of the remaining 112, 88 had submaxillary buboes (78·5%), 10 had inguinal buboes (8·9%), 9 had axillary buboes (8%), 3 had pelvic buboes (2·6%), 1 a submaxillary and an axillary bubo, 1 a pelvic and an axillary bubo.

present in the Sadar Bazaar. It was difficult to explain the appearance of this epizootic: it is suggestive, however, that a fortnight before the first plague rat in the Fort was caught, a bag of bran had been brought from a shop in the Sadar Bazaar situated in the centre of the epizootic area. The possibility of conveying rats and fleas in bags of grain cannot be denied. On two occasions in Belgaum, rats were seen escaping from bags of bran when these were being opened and on one occasion a nest of mice was found within a bran bag. As has been said the population of the Fort is almost exclusively European and the small epizootic there was not associated with any human plague cases.

Of the 130 acute plague rats 71 were caught in Belgaum City. Of these, 35 were caught in the Market or in its immediate vicinity; the remainder were more or less evenly distributed throughout the town. This fact is of remarkable significance and shows how severe the epizootic was in the neighbourhood of the Market and how comparatively mild it was in the rest of the town. During October, November and December 1324 rats were caught in the market and in three adjacent streets: of these rats 29 were plague infected, *i.e.* 2.2%. In the remainder of the town Shahapur and Sadar Bazaar during the same period 8528 rats were caught of which 55 were plague infected, *i.e.* 0.6%. Now the market and adjacent streets consist very largely of shops and godowns many of which are not used as dwelling houses at all. Of the 35 rats affected with acute plague caught in the Market, 22 were caught in grain godowns, stores, or grocers' shops (none of which were dwelling houses). In addition 10 rats presenting lesions of resolving or chronic plague were caught in these shops and godowns which lesions were proved to contain virulent plague bacilli. Over and above this number there were many more rats caught which presented lesions which we have good reasons to believe were the residue of an attack of acute plague (see article on resolving plague, p. 335 and maps).

The above figures can be taken as an indication that an epizootic of very considerable severity was raging in the neighbourhood of the Market at the height of the Belgaum epidemic. A reference has been made above to the weekly markets that are held in Belgaum at which not only the inhabitants of Belgaum itself but of all surrounding villages obtain their weeklystore. Given an epizootic such as we have seen raging in the Market it would be difficult to imagine more favourable conditions for the dissemination and spread of infection than this weekly gathering affords, a spread not only to other parts of Belgaum but to surrounding villages. The commission agents, such as those whose

stores, as we have seen above, were harbouring plague infected rats, import and export grain etc. by road and rail and sell it to retail and other wholesale dealers. The grain is contained in gunny bags which are capable of harbouring not only rat fleas but rats as well. It requires little imagination to picture what a serious danger a market such as that of Belgaum is, in times of plague, not only to itself but to all places with which it has trade intercourse. If there is one part of the town that is frequented by all the inhabitants, that part is the market. Godowns, in a market such as that of Belgaum, constructed as they are, and situated in the centre of the town, must always be a danger. The amount of food and shelter that they offer to rats is unlimited. There is always a danger of importation of plague infected rats or fleas or both in the sacks of grain and other stores that are continually being imported into these godowns and when once introduced with the large rat population that these buildings shelter there is nothing in the plague season to prevent the disease spreading and an epizootic and epidemic resulting.

V. SEASONAL PREVALENCE OF PLAGUE IN BELGAUM.

We have already stated that our first object in Belgaum was to ascertain what factors existed every year in July, August and September especially favourable to plague, which brought about the constant appearance of plague epidemics at this season of the year. Our year's observations have shown that these are the very months in which the rat flea is most prevalent. Whereas in the off plague season the average number of fleas per *Mus rattus* is as low as four to five, in these months the count reaches the large figure of eighteen. Further we have shown that the early months of the year are those in which the breeding activity of *Mus rattus* is at a maximum.

The decline of the epidemics in the months of December and January is in the same way associated with a decline in flea prevalence. In this connection Chart I is of interest. It was constructed as follows: the average number of deaths from plague, taking into account all the epidemics that Belgaum has suffered from (twelve years in all), was worked out for each week of the year (see Table IV). The curve so constructed has been superimposed on the curve that illustrates the seasonal prevalence of the rat flea in Belgaum during the year 1908—09. The time correlation that exists between flea prevalence and plague deaths is illustrated in a striking manner.

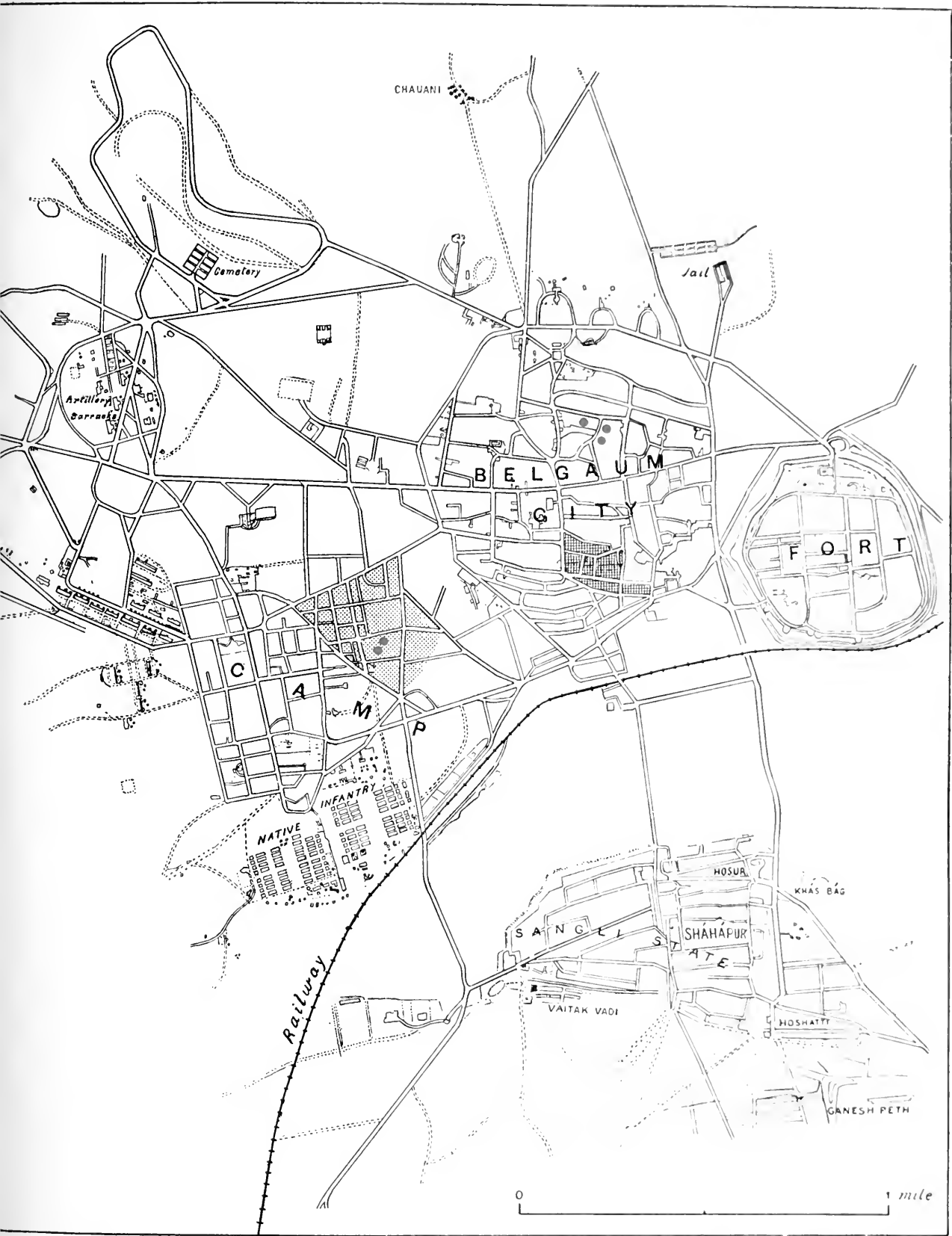
An insufficient number of rats may however be of more importance in bringing about the decline of an epidemic. This was illustrated by the little epidemic in the Sadar Bazaar which came to an end at a time when fleas were very numerous. On the other hand an insufficient number of fleas would appear to be the more important of the two factors in explaining the absence of epidemic plague in the off season, when rats may be numerous.

The third question to which we endeavoured to find a solution related to the fate of plague in the off season. We had evidence of the existence of acute rat plague in the off season in a village only three miles from Belgaum where the climatic conditions were in every way comparable to those of Belgaum. We also observed apparently indigenous human plague cases in Belgaum City in the middle of the off season, when climatic conditions were least favourable. These facts demonstrate the possibility of the off season being bridged over by dropping cases of acute plague amongst the rats. The epidemic which we studied apparently owed its origin to importation of infection. It must be remembered that the off plague season in Belgaum is the plague season in Bombay City and other parts of the Presidency, with which Belgaum has trade intercourse. It would be difficult, therefore, and often impossible, to definitely exclude the possibility of importation of infection in the study of any given epidemic of plague in Belgaum.

SUMMARY AND GENERAL CONCLUSIONS.

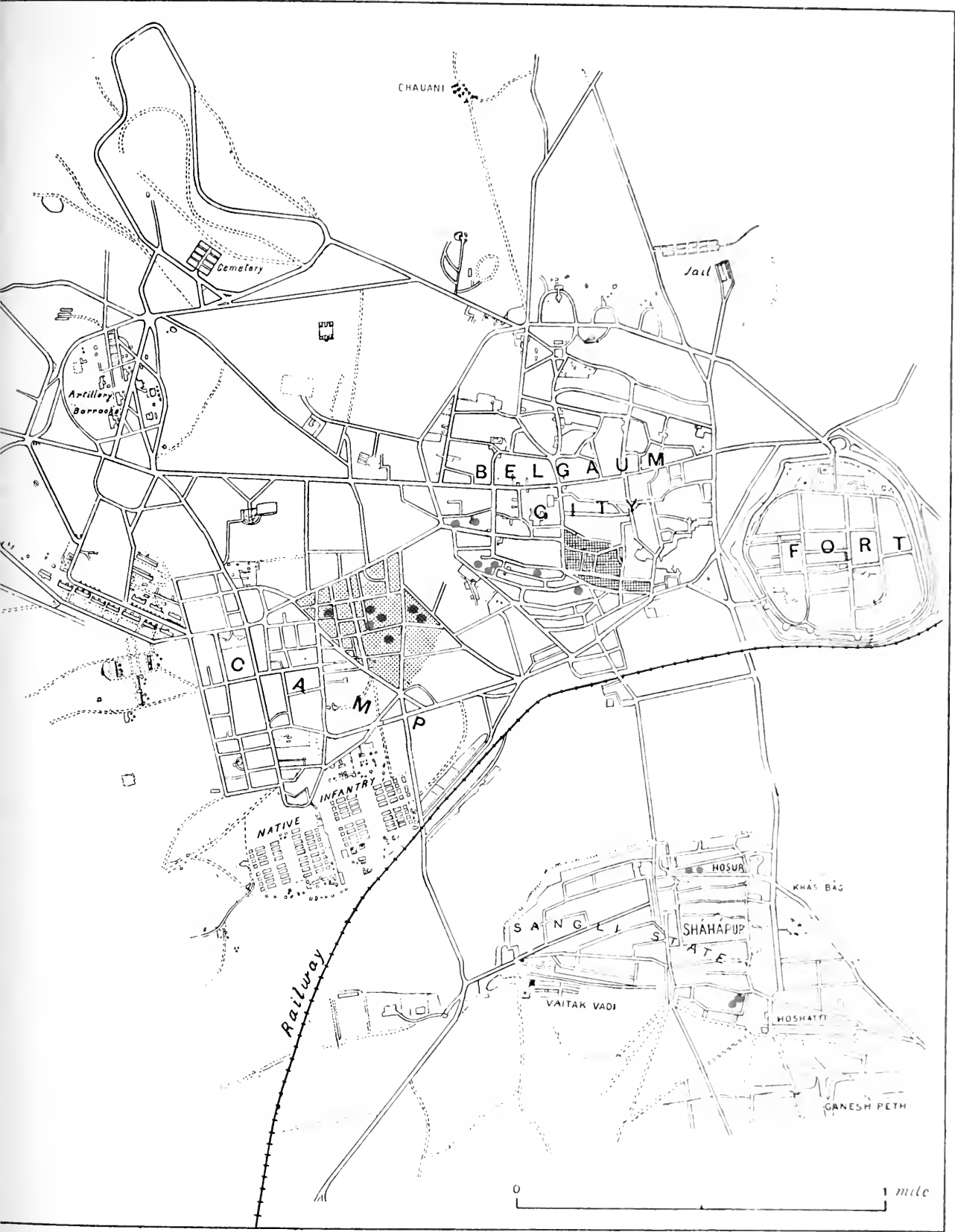
A study of all the epidemics of plague that have occurred in Belgaum from 1897 to 1909 shows that:

1. Plague cases can and do occur in any month of the year (see Table IV).
2. Though many of the cases that have occurred in the off plague seasons may have been imported, our observations have shown that indigenous cases (*i.e.* cases in which it was impossible to get a history of importation of infection) occur in the off plague season.
3. Though occasional cases may occur at any time of the year, plague can only assume epidemic proportions in the latter half of the year, July to November.
4. This seasonal prevalence is remarkably constant.
5. There has been a tendency for the epidemics to become progressively milder.



BELGAUM CITY AND ENVIRONS
Pre-epidemic period—May and June, 1908

● Human Plague Case



BELGAUM CITY AND ENVIRONS

July, 1908

- Human Plague Case
- Plague infected Rat (acute)

MAP IV

BELGAUM CITY AND ENVIRONS

August 1st—15th, 1908

● Human Plague Case
 ● Plague infected Rat (acute)
 ● Resolving Plague Rat

MAP IV

BELGAUM CITY AND ENVIRONS

August 1st—15th, 1908

- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat

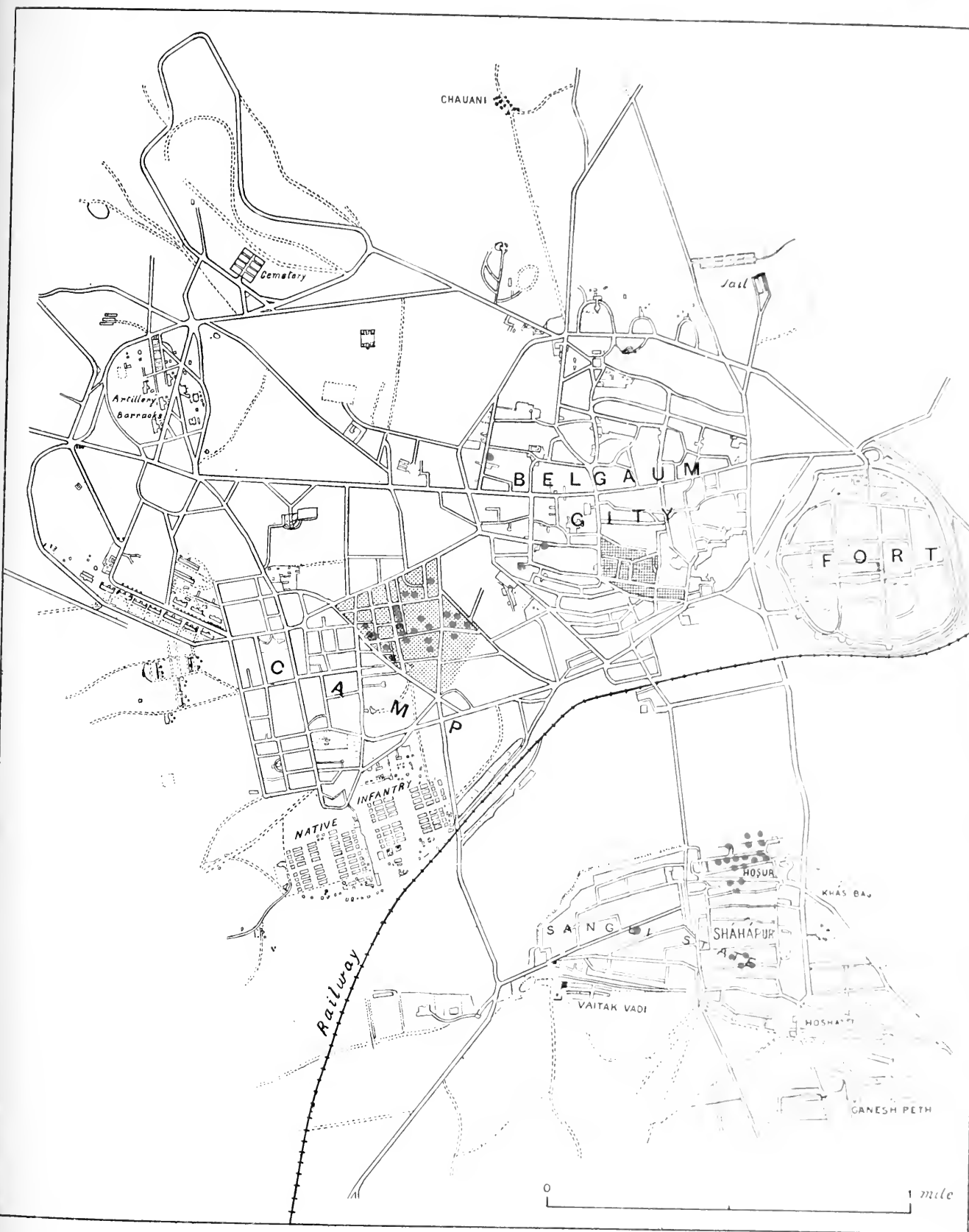
MAP IV

BELGAUM CITY AND ENVIRONS

August 1st—15th, 1908

- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat

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- MAP IV
- BELGAUM CITY AND ENVIRONS
- August 1st—15th, 1908
- Human Plague Case
 - Plague infected Rat (acute)
 - Resolving Plague Rat



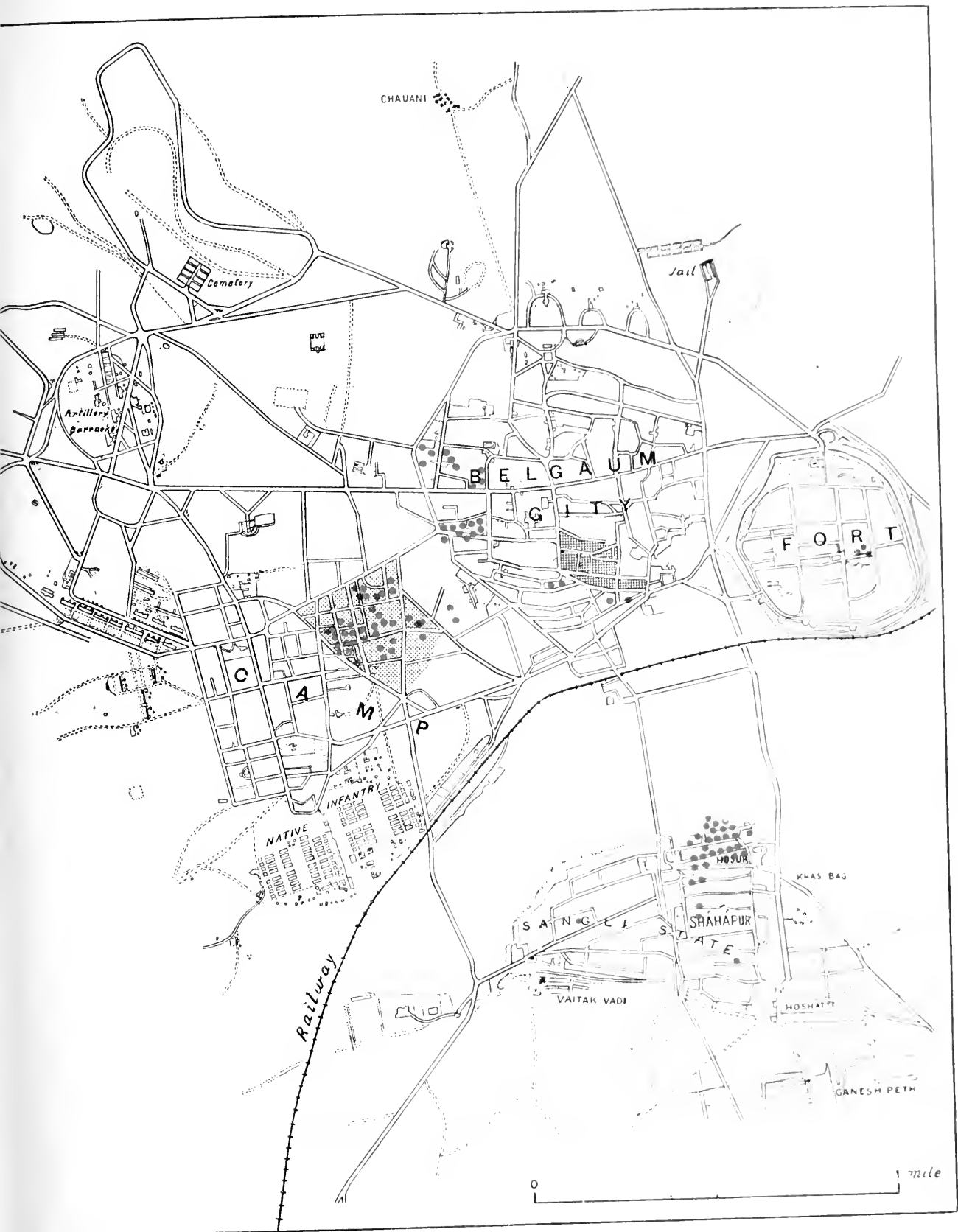
BELGAUM CITY AND ENVIRONS

August 16th—31st, 1908

- Human Plague Case
- Plague infected Rat (acute)



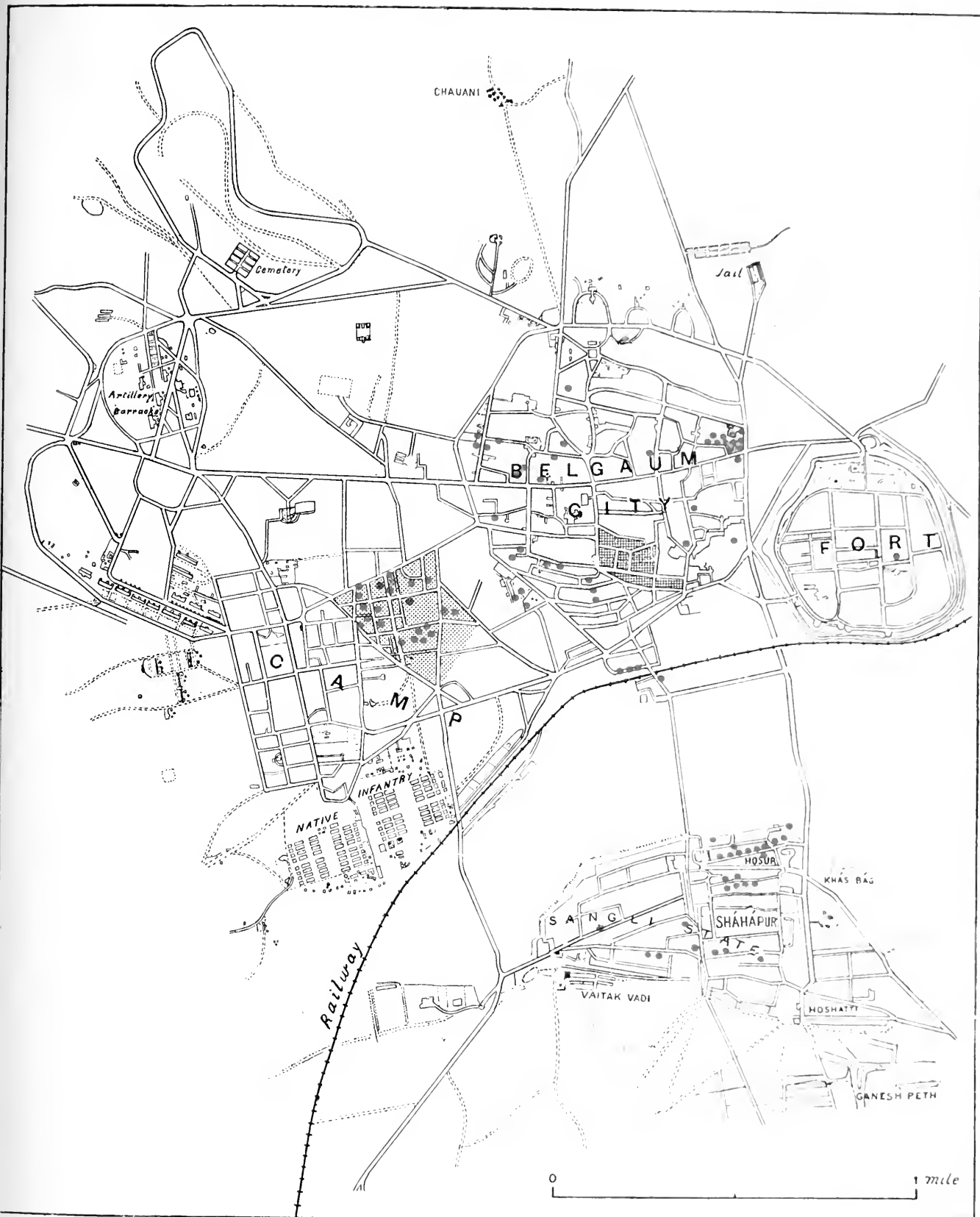
MAP VI



BELGAUM CITY AND ENVIRONS

September 1st—15th, 1908

- Human Plague Case
- Plague infected Rat (acute)



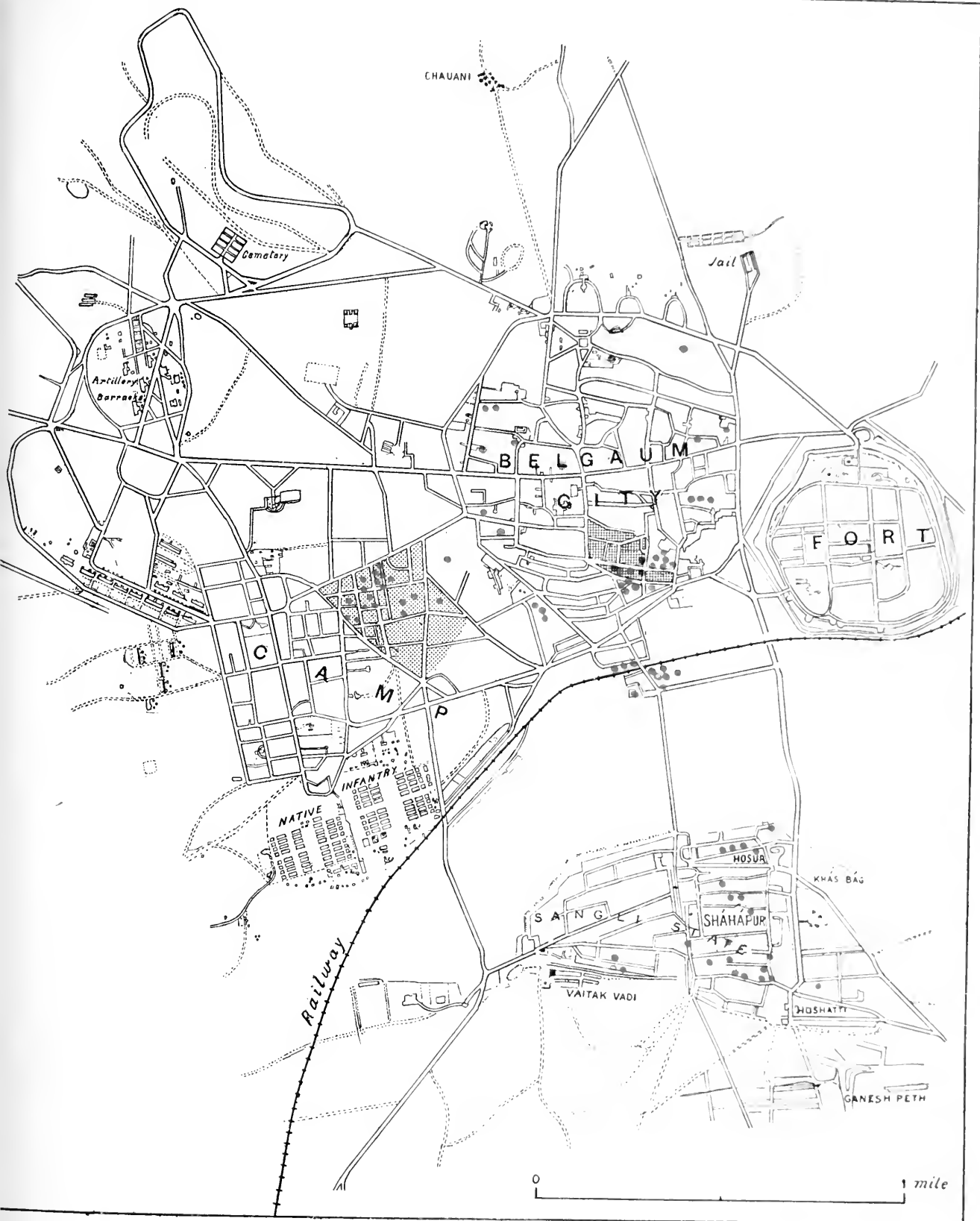
BELGAUM CITY AND ENVIRONS

September 16th—30th, 1908

- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat



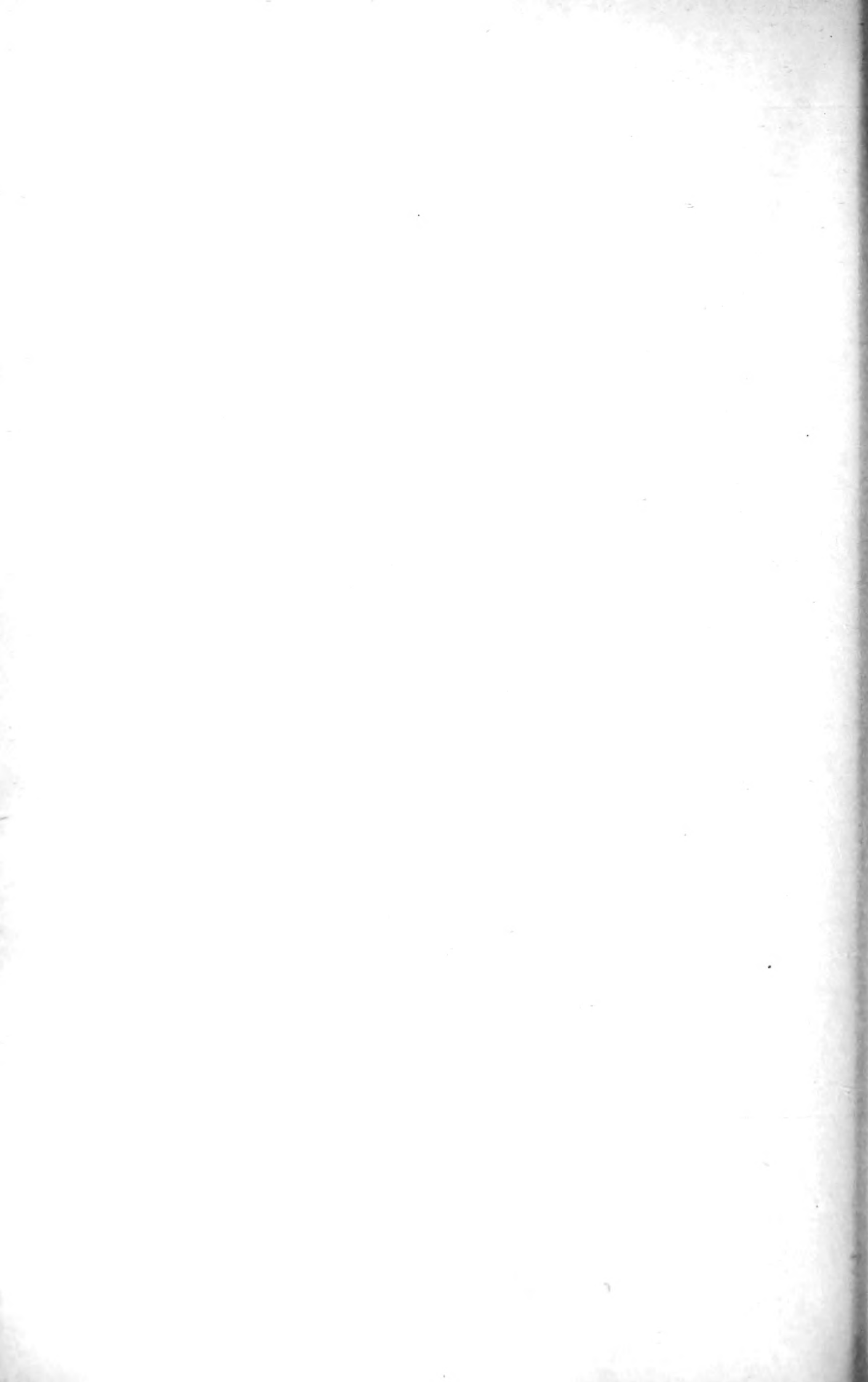
MAP VIII

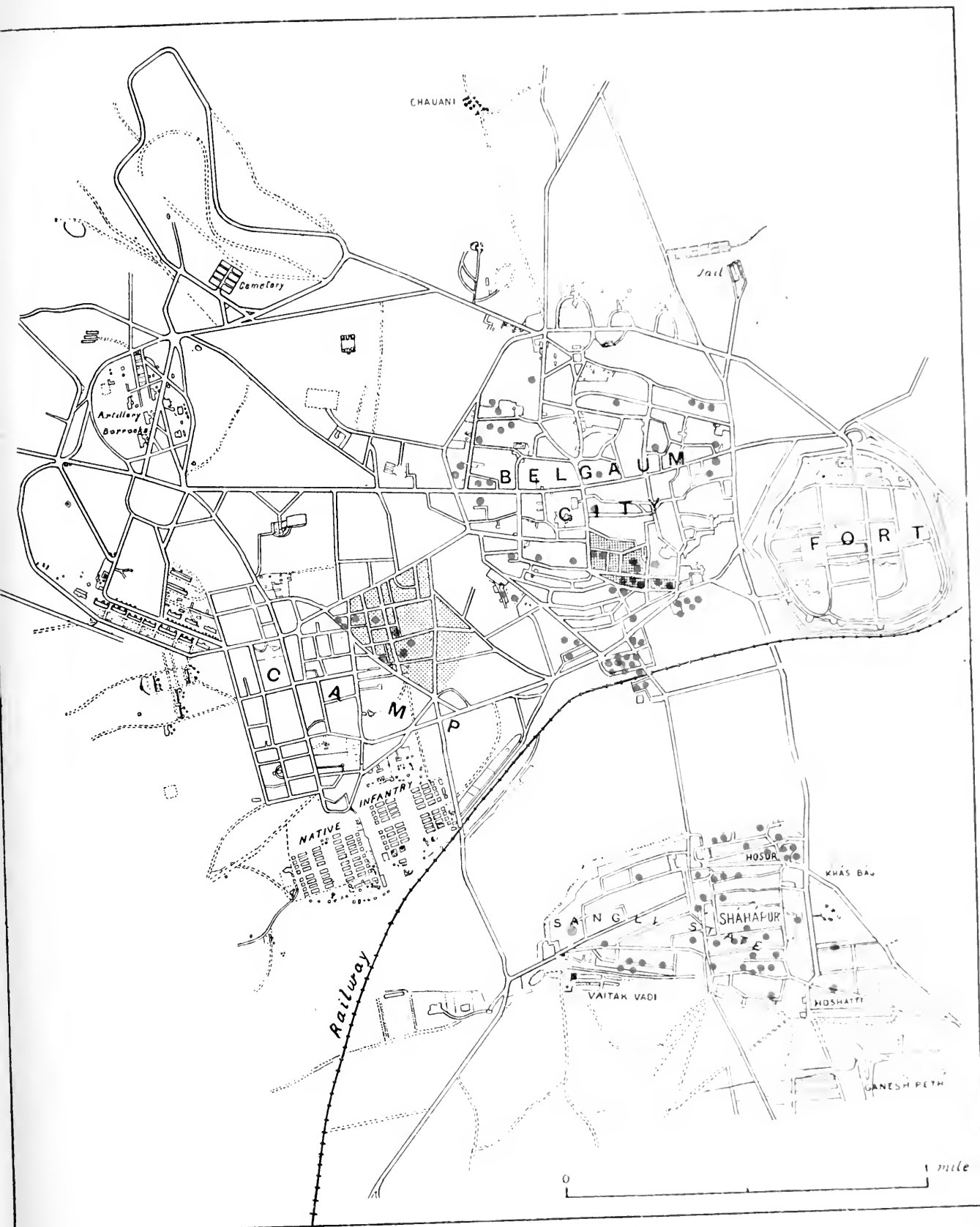


BELGAUM CITY AND ENVIRONS

October 1st—15th, 1908

- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat



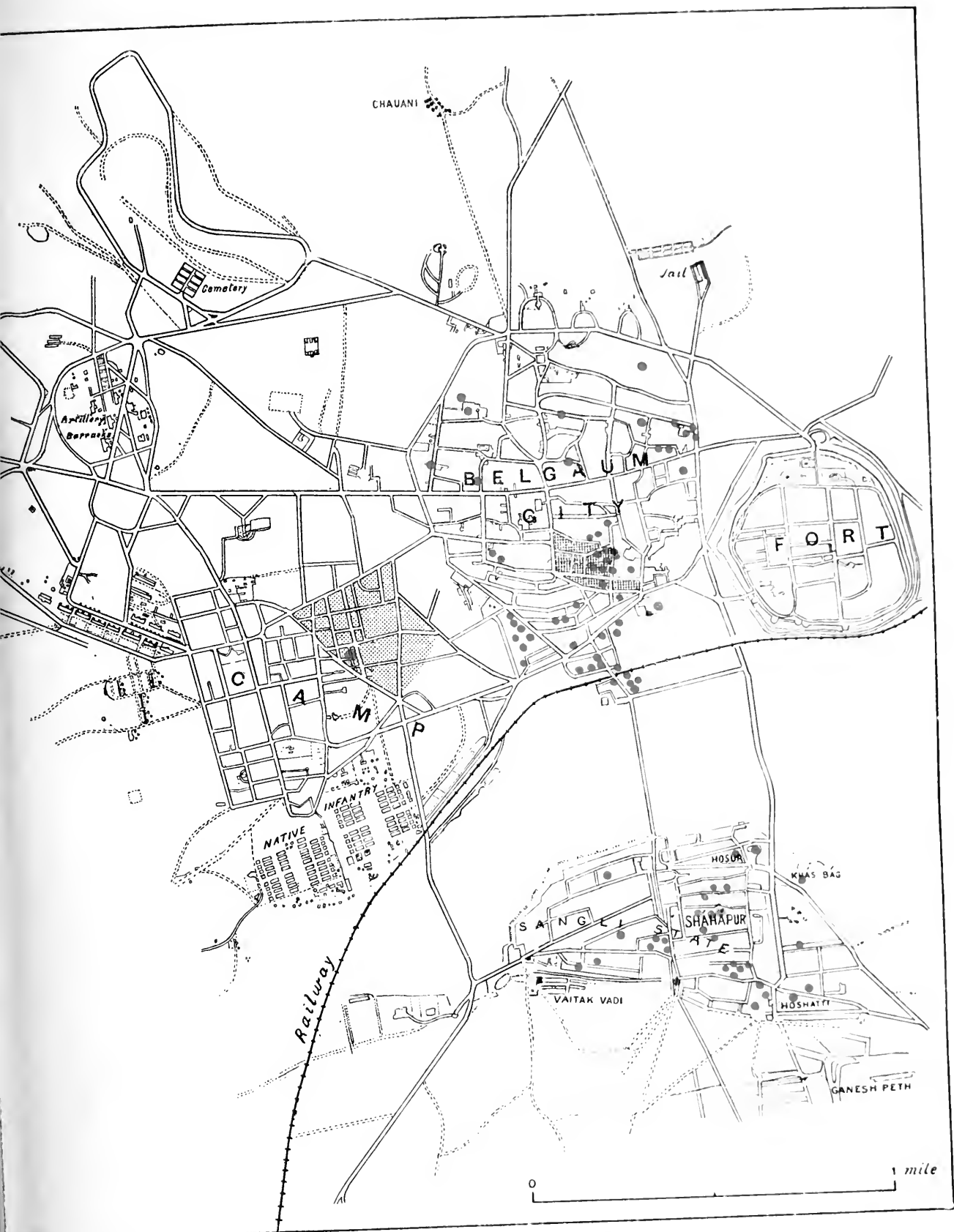


BELGAUM CITY AND ENVIRONS

October 16th—31st, 1908

- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat



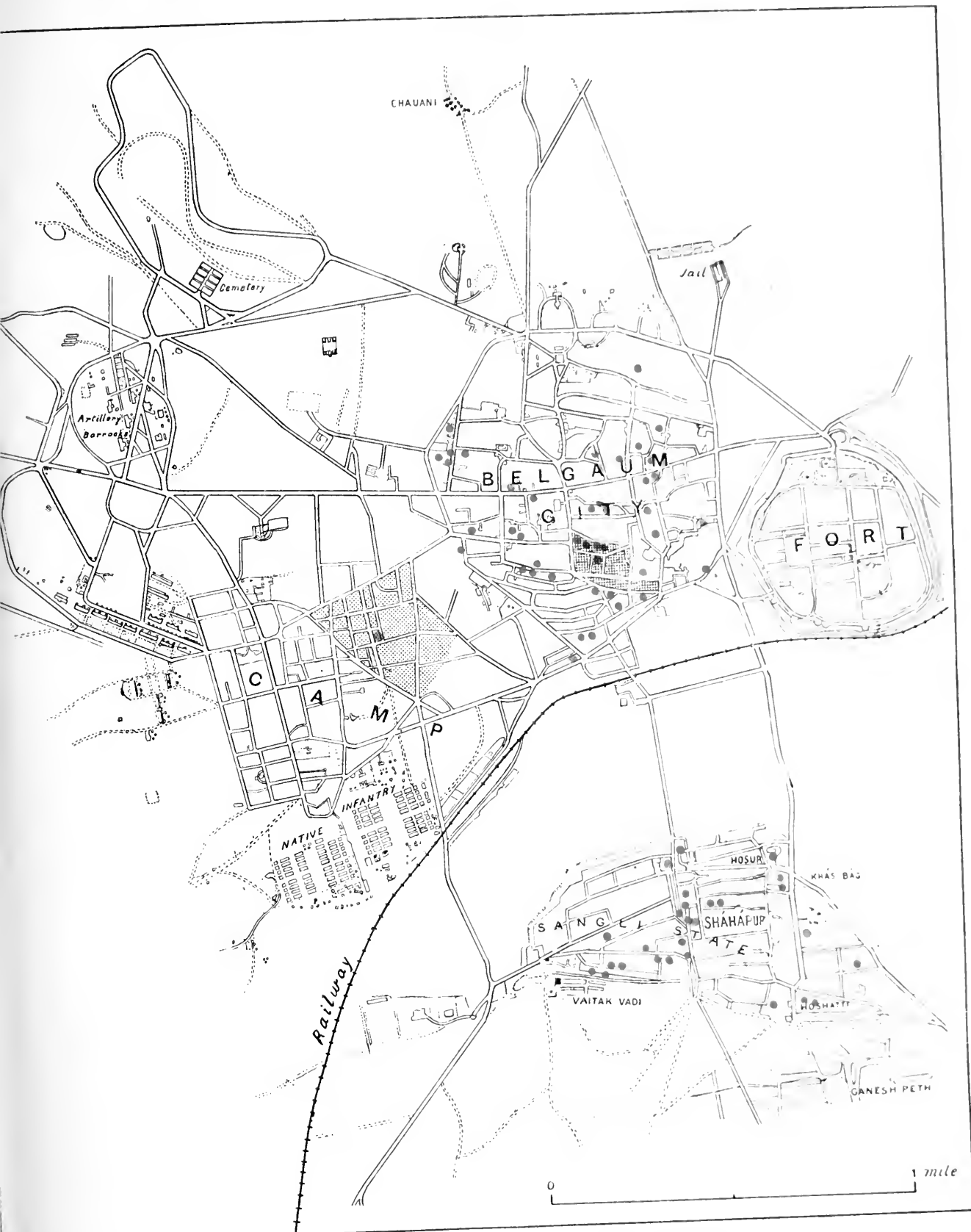


BELGAUM CITY AND ENVIRONS

November 1st—15th, 1908

- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat

MAP XI

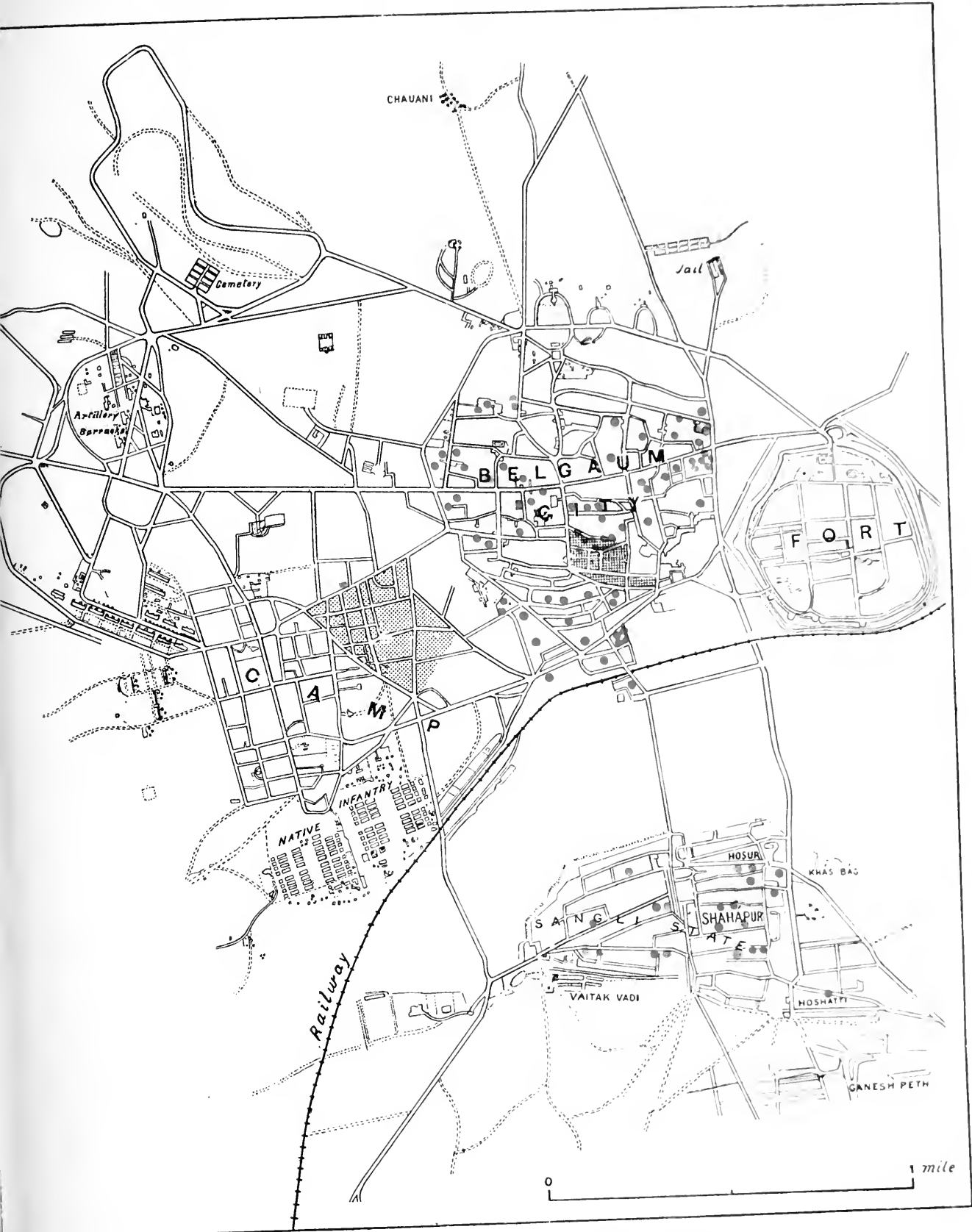


BELGAUM CITY AND ENVIRONS

November 16th—30th, 1908

- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat

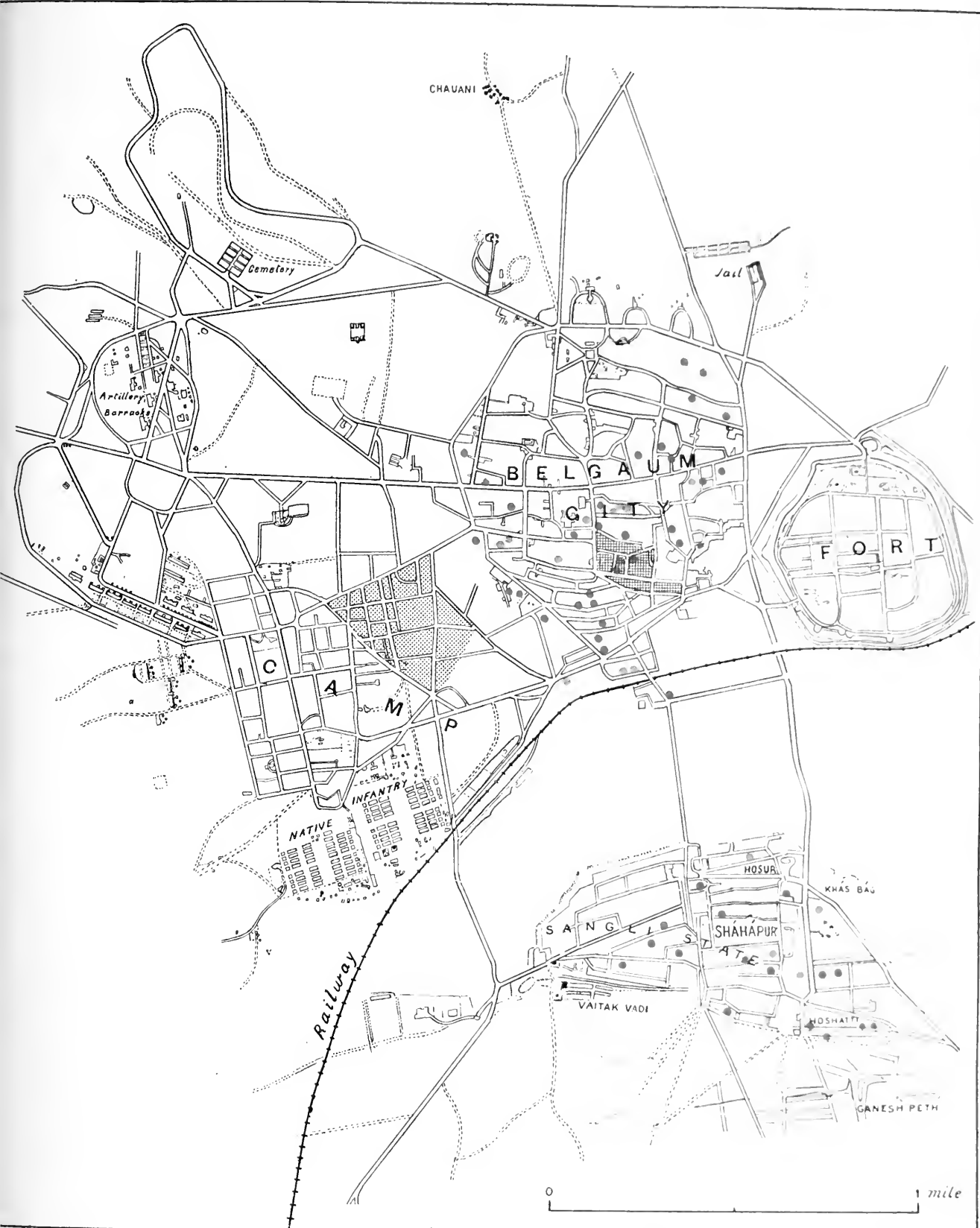




BELGAUM CITY AND ENVIRONS

December 1st—15th, 1908

- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat

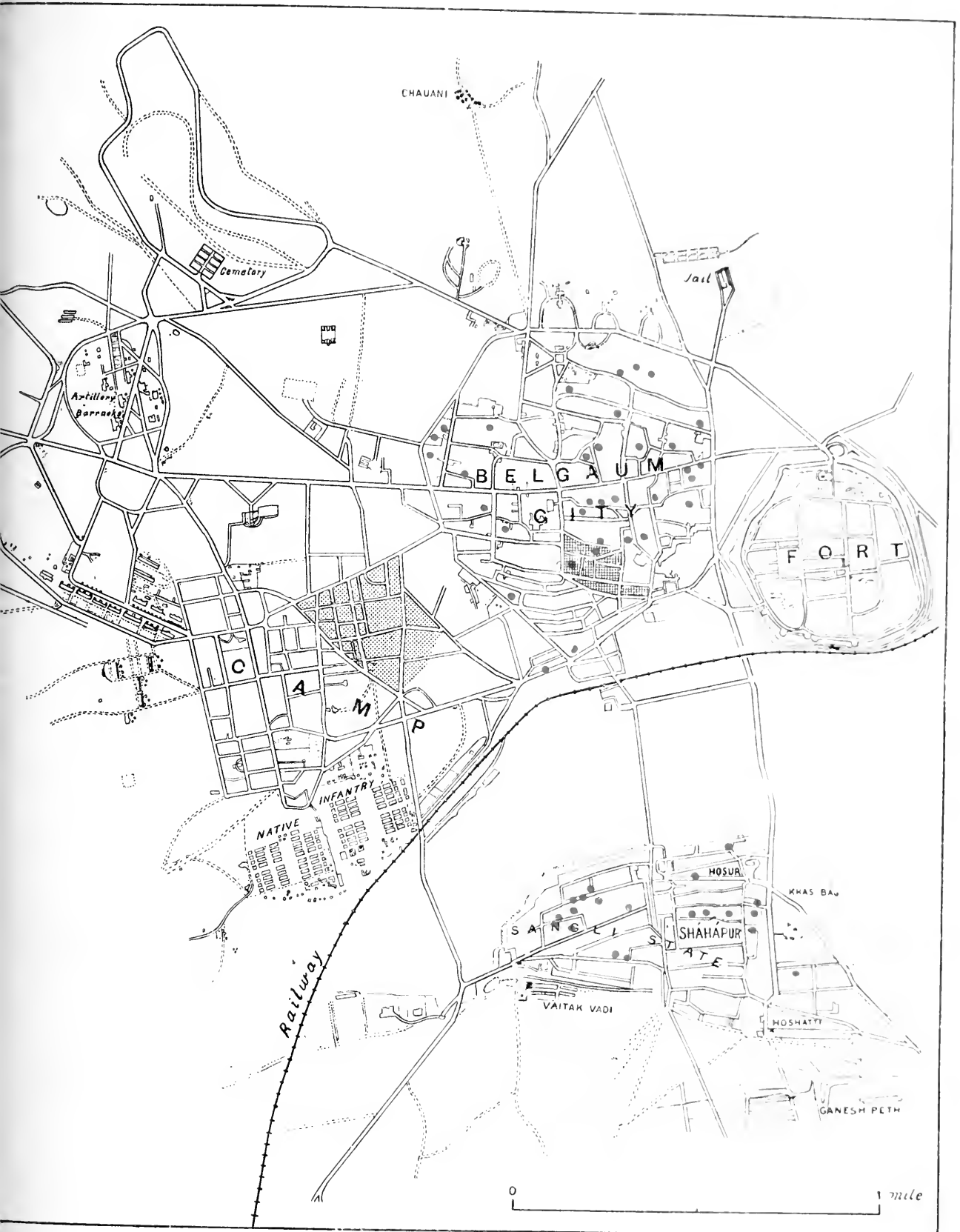


BELGAUM CITY AND ENVIRONS

December 16th—31st, 1908

- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat

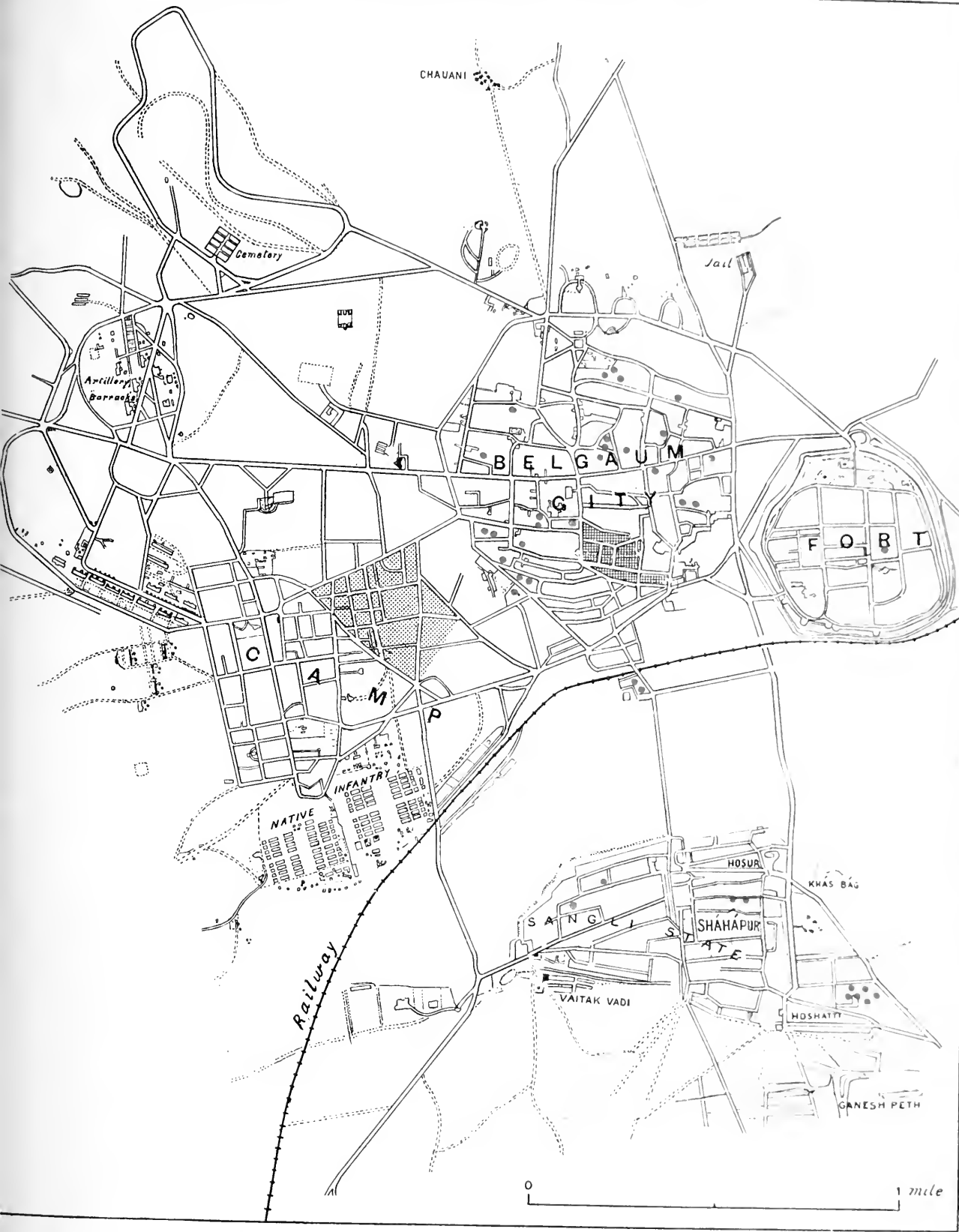




BELGAUM CITY AND ENVIRONS

January, 1909

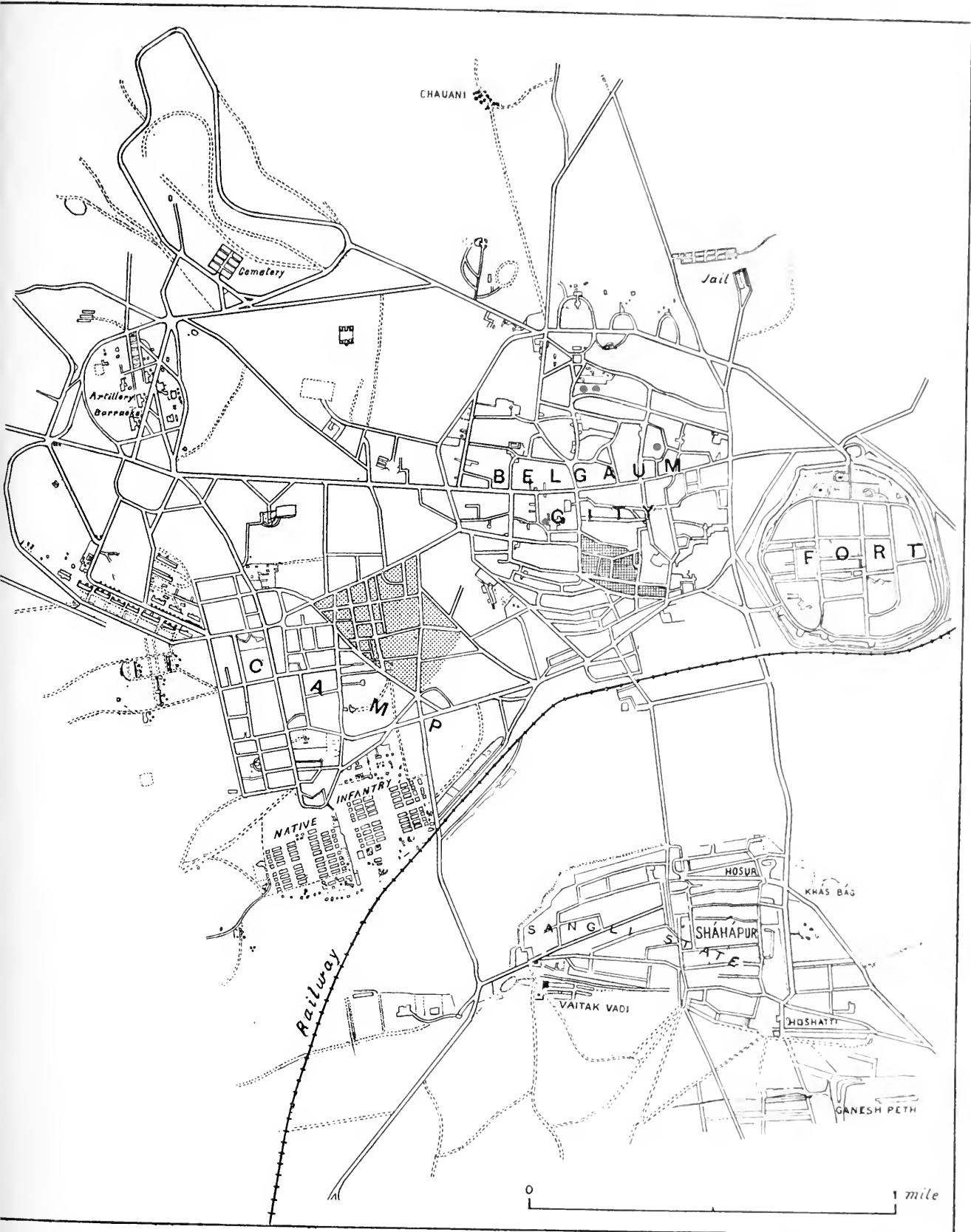
- Human Plague Case
- Plague infected Rat (acute)
- Resolving Plague Rat



BELGAUM CITY AND ENVIRONS

February, 1909

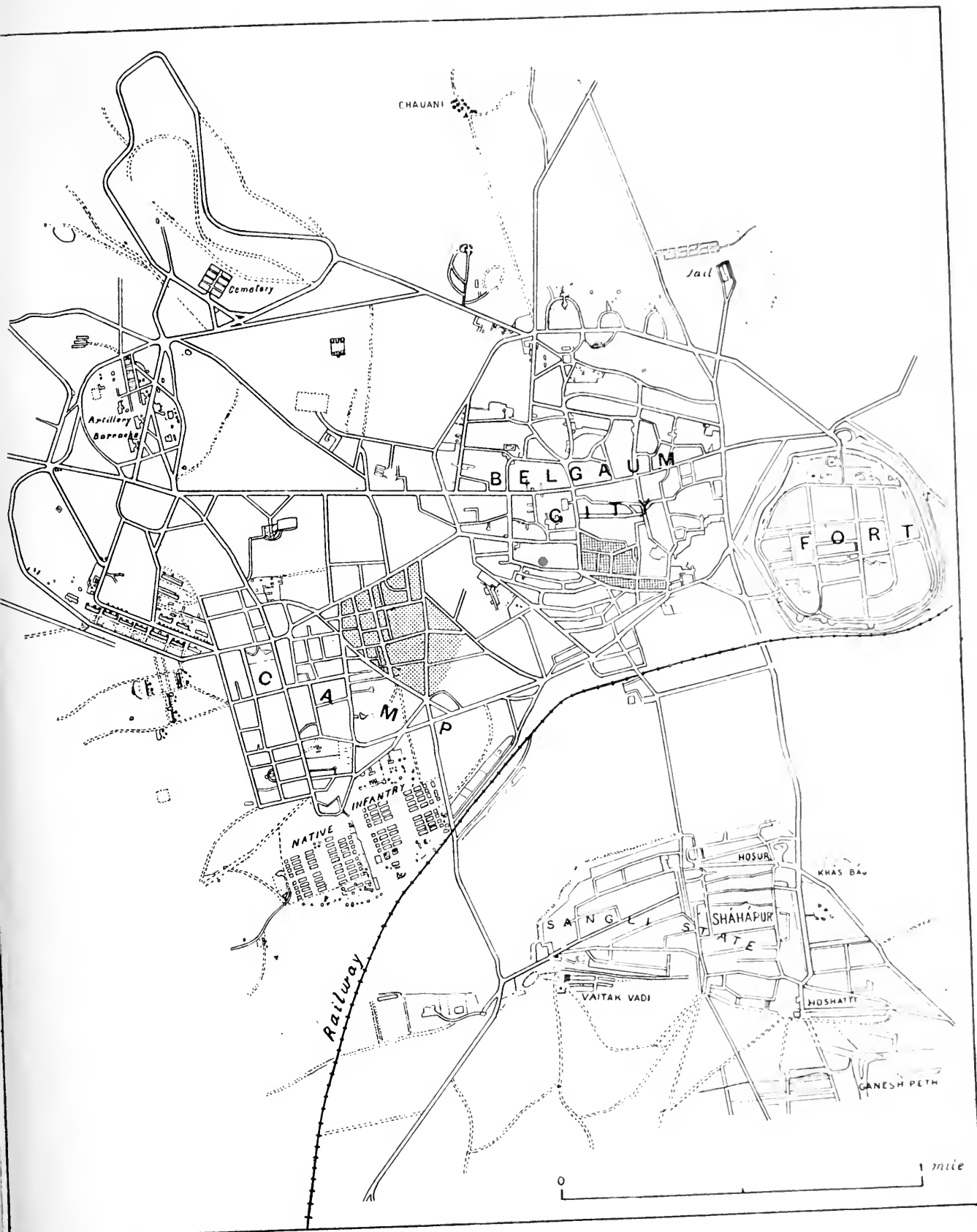
- Human Plague Case
- Plague infected Rat (acute)



BELGAUM CITY AND ENVIRONS

March, 1909

● Human Plague Case



BELGAUM CITY AND ENVIRONS

April, 1909

Our observations carried out in Belgaum in 1908—09 indicate :—

(A) That there is in Belgaum a definite seasonal variation in the prevalence of the rat flea, *Loemopsylla cheopis*.

(B) That this seasonal prevalence of the rat flea is closely correlated with the seasonal prevalence of plague.

(C) If it be granted that the seasonal prevalence of fleas is likely to be constant year after year, the height of each epidemic that has ever occurred in Belgaum was in a month of the year in which the rat flea was very prevalent.

(D) That there appears to be a close connection between the flea population and the hygrometric condition of the atmosphere. It would appear probable that the relation between the two is particularly close within certain limits of temperature.

(E) That the onset of an epidemic, if infection be present, follows and is determined by a rise in the number of rat fleas.

TABLE I.

Summary of Mus rattus caught and examined in Belgaum.
May 1908—June 1909.

Week ending	Total no. of rats examined	No. of rats on which fleas were counted	Total no. of fleas	Average no. of fleas per rat	Total no. of adult females	Total no. of pregnant females	Percentage of adult females pregnant	Average no. of foetuses	Total no. of young rats (70 gms. and under)	Percentage young of total	No. of rats per 100 traps set
May 16 '08	211	211	721	3.4	78	30	38.4	6.0	70	33.1	30.5
23	412	412	1975	4.7	155	91	58.7	5.1	137	33.2	29.1
30	829	829	3931	4.7	280	174	62.1	5.6	330	39.8	35.4
June 6	887	788	4606	5.8	300	167	55.6	5.6	341	38.4	36.6
13	636	621	4653	7.4	192	104	54.1	5.2	220	34.4	26.1
20	718	718	4955	6.9	253	135	53.3	5.3	254	35.3	28.9
27	834	834	7839	9.4	277	128	46.2	5.2	325	38.9	33.3
July 4	771	656	7120	10.9	265	98	37.0	5.3	283	36.7	31.2
11	1037	1035	12794	12.4	327	182	55.6	5.7	355	34.2	35.4
18	1006	706	12003	17.0	360	178	49.4	5.9	336	33.4	34.6
25	890	756	13940	18.4	310	182	58.7	5.7	324	36.4	33.1
Aug. 1	984	902	15745	17.5	317	130	41.0	5.6	371	37.7	35.6
8	905	762	13235	17.4	340	151	44.4	6.0	302	33.3	29.3
15	835	804	11968	14.9	319	95	29.7	5.3	303	36.2	28.2
22	933	886	13987	15.8	345	92	26.6	5.5	275	29.5	30.7
29	811	795	12534	15.8	259	93	35.9	6.0	275	33.9	27.4
Sept. 5	655	630	10127	16.0	226	80	35.4	5.7	253	38.6	24.0
12	957	924	17030	18.4	305	88	28.8	5.1	358	37.4	32.6
19	954	888	15514	17.4	303	81	26.7	5.5	374	39.2	33.1
26	865	841	12751	15.2	253	36	14.2	5.2	291	33.6	29.9
Oct. 3	774	741	13816	18.6	265	67	25.3	5.3	259	33.5	26.8
10	759	736	12243	16.6	211	61	28.9	5.4	326	42.9	26.0
17	816	782	11725	15.0	252	86	34.1	5.4	311	38.1	28.1
24	916	896	11651	13.0	327	100	30.6	5.3	326	35.4	31.3
31	838	820	10854	13.2	289	58	20.0	5.1	294	35.0	28.3
Nov. 7	699	696	9913	14.2	215	44	20.5	5.0	274	39.2	24.4
14	777	764	11449	15.0	239	46	19.2	5.0	307	39.5	26.5
21	692	674	9756	14.5	197	66	33.5	5.0	261	38.7	23.8
28	750	741	7860	10.6	250	67	26.8	4.8	243	32.4	25.8
Dec. 5	724	698	7077	10.1	232	72	31.0	5.3	221	30.5	25.3
12	602	592	4914	8.3	164	48	29.3	5.4	244	40.5	21.5
19	582	568	4953	8.7	175	39	22.25	5.2	183	31.4	20.4
26	592	573	3127	5.4	196	56	28.5	5.3	188	30.7	24.0
Jan. 2 '09	461	449	2850	6.3	155	56	36.1	5.0	139	30.1	18.5
9	343	337	1889	5.6	114	31	27.2	5.3	99	29.0	14.1
16	461	404	2379	5.8	158	33	20.9	5.3	141	30.6	16.6
23	423	404	1978	4.9	149	72	48.3	5.0	118	27.9	14.2
30	451	442	2968	6.7	144	76	52.8	5.6	163	36.0	15.2
Feb. 6	392	372	2749	7.4	120	56	46.6	5.1	145	37.0	13.2
13	422	412	3724	9.0	122	76	62.3	5.3	183	43.3	14.2
20	420	417	3247	7.7	101	72	71.2	5.4	203	48.3	17.1
27	498	490	3378	6.9	121	65	53.7	5.7	223	44.7	16.6
Mar. 6	422	412	2723	6.6	105	54	51.4	5.2	208	49.3	15.2
13	370	356	1872	5.25	89	49	55.0	5.1	202	54.6	13.6
20	462	460	2114	4.6	132	63	47.7	5.5	193	41.7	16.6
27	439	436	3048	7.0	109	54	50.0	5.8	227	51.7	15.6
April 3	483	474	2759	5.8	123	59	48.0	5.4	222	46.0	15.2
10	480	474	2806	5.9	126	59	46.8	5.1	255	53.1	14.0
17	486	477	2738	5.7	120	59	49.1	5.4	234	48.1	13.9
24	633	627	3144	5.0	162	79	48.8	5.9	306	48.3	17.7
May 1	585	575	2373	4.1	119	41	34.4	5.5	314	53.6	16.0
8	559	543	2049	3.7	124	65	52.4	5.2	301	53.8	15.0
15	554	539	1945	3.6	154	57	37.0	5.5	246	44.4	15.2
22	535	533	2131	4.0	123	80	65.0	5.2	257	48.0	14.7
29	561	547	2151	3.9	150	65	43.3	4.6	293	52.2	15.4
June 5	591	580	3230	5.5	154	111	72.0	5.0	282	47.0	16.4
12	591	579	3163	5.4	147	82	55.7	5.0	287	48.5	16.6
19	640	619	4626	7.4	160	119	74.3	5.2	290	45.3	18.0
26	671	653	5878	9.0	167	83	49.7	4.8	346	51.5	22.0

TABLE II.
Belgaum. 1908—1909.

Week ending	Mean temperature	Humidity	Wet bulb reading
May 23 '08	78·8	68·8	72·5
30	79·0	67·5	72·0
June 6	79·0	68·8	72·5
13	77·5	75·8	72·5
20	73·0	86·3	70·5
27	74·5	81·3	71·0
July 4	71·8	90·1	70·0
11	71·2	91·4	69·5
18	70·5	92·7	69·25
25	71·0	90·5	69·3
Aug. 1	71·0	93·6	70·0
8	71·1	93·0	70·0
15	70·8	89·4	69·0
22	71·1	89·4	69·0
29	70·8	89·5	69·0
Sept. 5	72·5	83·6	69·5
12	72·0	79·2	68·0
19	72·5	83·6	69·5
26	73·2	84·8	70·0
Oct. 3	74·0	76·3	69·5
10	74·6	69·4	68·5
17	75·2	69·7	69·25
24	74·5	71·0	68·5
31	75·0	77·0	70·5
Nov. 7	73·7	69·5	67·0
14	68·9	66·1	62·5
21	66·0	53·8	56·6
28	67·7	63·3	60·7
Dec. 5	66·9	51·7	57·0
12	66·3	58·3	57·7
19	65·2	58·8	57·0
26	66·8	66·6	62·5
Jan. 2 '09	68·3	65·6	61·5
9	71·5	69·1	65·5
16	68·4	66·7	62·0
23	70·0	64·0	62·7
30	71·9	59·8	63·7
Feb. 6	68·6	56·0	59·5
13	72·5	59·3	64·7
20	72·9	62·1	65·0
27	79·3	59·8	70·5
Mar. 6	78·4	49·5	66·5
13	78·5	56·3	68·5
20	79·4	56·4	69·5
27	79·3	59·8	70·5
April 3	79·2	57·0	69·3
10	79·2	53·5	68·0
17	79·7	53·7	68·7
24	78·3	63·0	70·0
May 1	82·2	60·2	73·0
8	80·5	67·7	73·5
15	78·3	70·5	72·0
22	81·2	65·5	73·5
29	80·5	66·2	73·5
June 5	75·0	74·0	70·0
12	72·5	88·0	70·5
19	73·0	81·5	69·5
26	73·0	83·0	70·0

The "mean" temperature in this table was calculated from the daily maximum and minimum readings. It is therefore not accurately speaking a true mean temperature. It has been found however in the case of Belgaum that the result so obtained does not differ much from the true mean temperature calculated from hourly readings (as well as by the use of Schell's formula) by more than two-thirds to one degree. The humidity was worked out in a similar way.

TABLE III.

Plague cases and deaths in Belgaum during 1908—1909.

Week ending	City*		(Sadar Bazaar) ¹ Cantonment		Shahapur		Total	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
June 6 '08	1	1	2	0	0	0	3	1
13	2	2	0	0	0	0	2	2
20	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
July 4	0	0	0	0	0	0	0	0
11	0	0	1	0	0	0	1	0
18	3	3	0	1	0	0	3	4
25	4	3	0	0	0	0	4	3
Aug. 1	2	0	0	0	0	0	2	0
8	3	2	2	1	5	4	10	7
15	2	0	3	2	5	5	10	7
22	5	3	8	6	2	1	15	10
29	10	7	11	11	5	1	26	19
Sept. 5	14	12	11	8	5	4	30	24
12	20	14	17	13	2	2	39	29
19	26	16	16	9	3	0	45	25
26	23	13	10	10	1	1	34	24
Oct. 3	26	16	11	7	8	2	45	25
10	18	12	7	5	4	4	29	21
17	8	6	7	3	8	4	23	13
24	20	16	4	2	21	7	45	25
31	33	21	1	1	13	11	47	33
Nov. 7	27	21	1	0	9	7	37	28
14	43	23	1	1	9	8	53	32
21	23	14	0	0	9	5	32	19
28	15	9	0	0	5	4	20	13
Dec. 5	32	19	0	0	10	6	42	25
12	20	12	1	0	3	1	24	13
19	23	17	0	0	5	5	28	22
26	16	11	0	0	5	5	21	16
Jan. 2 '09	13	10	0	0	4	3	17	13
9	11	10	0	0	6	5	17	15
16	11	6	0	0	1	1	12	7
23	7	5	0	0	5	1	12	6
30	4	3	0	0	3	3	7	6
Feb. 6	14	7	0	0	2	1	16	8
13	8	5	0	0	1	2	9	7
20	8	6	0	0	0	0	8	6
27	5	2	0	0	2	1	7	3
Mar. 6	3	0	0	0	0	1	3	1
13	1	1	0	0	1	0	2	1
20	2	2	0	0	0	0	2	2
April 17	1	1	0	0	0	0	1	1
Total	507	331	114	80	162	105	783	516

* Municipal area including suburbs.

TABLE IV.

Plague deaths in Belgaum—week by week—for the years 1897—1909.

Week ending	1897	98	99	1900	01	02	03	04	05	06	07	08	09	Totals
Jan. 2	—	49	8	0	1	6	3	9	9	0	0	10	10	105
9	—	63	7	1	0	2	6	10	6	4	0	7	10	116
16	—	59	7	4	0	1	6	8	3	7	0	4	6	105
23	—	48	2	5	0	3	7	7	6	4	0	7	5	94
30	—	39	4	2	0	4	10	5	5	4	0	3	3	79
Feb. 6	—	23	4	2	0	3	8	12	6	4	0	6	7	75
13	—	12	8	2	0	0	3	1	7	6	0	4	5	48
20	—	6	6	2	1	1	3	2	1	9	0	8	6	45
27	—	12	3	3	1	0	5	2	3	4	0	7	2	42
Mar. 6	—	10	7	2	1	1	4	2	6	5	0	13	0	51
13	—	7	2	3	0	0	5	3	2	4	0	3	1	30
20	—	0	1	0	1	1	1	2	1	8	0	1	2	18
27	—	7	6	4	1	0	0	3	1	3	0	1	0	26
April 3	—	5	2	4	1	1	1	0	1	1	0	1	0	17
10	—	0	1	2	0	0	4	0	0	1	0	3	0	11
17	—	1	4	2	0	1	1	1	0	2	0	0	1	13
24	—	2	8	6	0	0	4	0	0	0	0	0	0	20
May 1	—	1	9	8	0	0	0	0	0	0	0	0	0	18
8	—	2	11	2	1	0	0	2	0	0	0	0	0	18
15	—	2	14	1	5	0	2	0	0	0	0	0	0	24
23	0	2	34	1	0	1	0	1	0	0	0	0	—	39
30	0	0	41	7	0	0	0	1	1	2	0	0	—	52
June 6	0	0	43	6	0	1	0	0	0	1	0	1	—	52
13	0	1	42	2	0	0	2	0	0	0	0	2	—	49
20	0	8	54	4	1	0	1	0	0	0	0	0	—	68
27	0	3	59	2	0	1	4	0	1	0	0	0	—	70
July 4	0	12	97	3	0	2	2	1	1	0	0	0	—	118
11	0	11	117	7	0	0	9	0	0	1	0	0	—	145
18	0	52	160	5	3	4	7	2	3	0	0	4	—	240
25	0	74	183	8	3	0	5	4	2	0	0	3	—	282
Aug. 1	0	80	214	22	7	0	5	2	0	0	0	0	—	330
8	0	61	226	20	5	2	2	6	5	0	0	3	—	330
15	0	64	193	16	16	5	9	6	7	0	0	2	—	318
22	0	68	134	57	23	16	19	6	2	0	0	9	—	334
29	0	68	118	97	29	20	21	12	8	0	0	18	—	391
Sept. 5	0	82	110	130	40	27	42	17	11	0	0	20	—	479
12	0	136	81	157	51	35	43	32	13	0	0	27	—	575
19	0	147	35	175	67	70	65	32	12	1	0	25	—	629
26	0	180	23	184	90	114	81	44	15	1	2	23	—	757
Oct. 3	0	255	6	177	87	118	90	65	15	1	1	23	—	838
10	0	261	6	129	95	128	99	58	17	1	0	17	—	811
17	0	274	9	131	89	164	88	61	16	0	3	9	—	844
24	0	238	3	86	77	149	84	47	21	0	4	18	—	727
31	5	148	5	38	86	136	83	98	11	0	2	22	—	634
Nov. 7	20	91	4	16	75	88	62	69	20	1	7	21	—	474
14	14	90	0	8	44	88	44	51	21	0	9	24	—	393
21	21	60	6	4	52	63	63	41	14	0	14	14	—	352
28	38	47	5	10	25	55	27	33	14	0	14	9	—	277
Dec. 5	61	19	4	5	21	43	23	34	21	0	6	19	—	256
12	39	24	3	5	15	31	20	26	16	0	4	12	—	195
19	36	15	1	3	5	18	12	21	9	0	11	17	—	148
26	52	13	0	1	5	17	10	7	13	0	9	11	—	138
Total	286	2932	2130	1571	1024	1420	1095	846	346	75	86	431	58	12300

APPENDIX.

A census of men and animals in Belgaum.

Towards the close of our observations in Belgaum, a census was made of the City and the Sadar Bazaar Camp.

A few notes were made of the structure of each house, the number of inmates, and the caste, occupation and trade of the inhabitants. In addition notes were made of the number of animals, buffaloes, bullocks, cows, ponies, goats, dogs, cats, chickens that shared with their owners the shelter of their abodes. Further information as to the number of rats that had been caught in each house was filled in from our laboratory records. A note was also made against each house as to the number of plague cases and deaths that had occurred therein during the epidemic that we had had under observation.

We hoped that we might be able to learn something from the mass of facts so collected about the influence that the various habits and mode of living of the people had on the degree of infestation of their houses with rats. No facts of any great practical value or importance have come to light, nor have the results been at all commensurate with the great labour involved.

In Belgaum City (excluding suburbs) there are 4927 houses; of this number 3813 are inhabited, *i.e.* dwelling houses; 1114 are uninhabited, *i.e.* they are shops, godowns or stores, or else are dwelling houses that were empty or locked at the time of our visit. The human population was 23,885, which gives an average of 6.2 persons per inhabited house.

In the Sadar Bazaar there are 642 inhabited houses, and 151 uninhabited. The population is 3507, an average of 5.4 persons per house.

The houses in Belgaum are all numbered; it occasionally happened however that more than one house bore the same number; this fact as well as other errors in numbering rendered it occasionally impossible for us to subsequently identify houses in which we had previously caught rats: this did not occur sufficiently often however as to in any way vitiate our general results.

During the fourteen months that our observations lasted in Belgaum City only 20,684 rats were caught in 2422 houses, an average of 8.5 rats per house. In this calculation we have excluded all houses in which we failed to catch one or more rats. The cause of our failure to catch any

rats after repeated trial in any given house was in the vast majority of cases attributable to the unwillingness of the inmates to help us. They either placed the traps in places inaccessible to rats, or else liberated the rats that had been caught. We assume that efficient trapping will succeed in catching at least one rat a year in every house in Belgaum. During the twelve months observations in the Sadar Bazaar 3077 rats were caught in 370 houses, an average of 8·3 rats per house.

Of the inhabited houses in the city we found that 1128 harboured either buffaloes, bullocks, cows, ponies or goats. The number of such animals was 3208, an average of nearly three per house. These figures will give some idea of the close association with domestic animals in which the Indian lives. In 623 houses that harboured one or more such animals, 5738 rats were caught, an average of 9·2 rats per house. In 1799 houses that contained no such animals 14,946 rats were caught, an average of 8·3 rats per house. In the Sadar Bazaar Camp, 402 rats were caught in 42 houses that harboured some of these larger domestic animals, in addition to their human inmates—an average of nearly ten rats per house; whereas in 328 houses that contained no such animals, 2675 rats were caught, an average of eight rats per house. Thus it would appear that the common custom of keeping such domestic animals in dwelling houses favours rat infestation but not to any very marked degree. The extra food supply available for rats in such houses does not appear to have the marked influence that one would anticipate.

There was only one house in Belgaum in which more than one hundred rats were caught in the course of the year; this was a grocer's shop in the City Market. It was a small house of three rooms, occupied by six people, Mahomedans. In this one house alone one hundred and ninety-seven rats were caught. It is a curious fact that this house possessed two cats. There were seventeen houses in which more than fifty and less than a hundred rats were caught; nine of these were ordinary dwelling houses, two were grocers' shops, one a grain godown, two liquor shops, one butcher shop, one stable, and one weaver's house. Two hundred and thirty-seven houses yielded a catch of more than twenty but less than fifty rats. Twenty-four of these were grocers' shops, sweet-meat sellers or grain stores, nineteen were weavers, eight were tailors' shops. No other trade seems to possess any influence especially favourable to rat infestation.

Cats and rat infestation.

Endeavours were made to get as accurate a cat census as possible of the city and the Sadar Bazaar. It was hoped that we should be able to get some figures that would express the value of the cat as a means of keeping down the rat population. We will state at once that the difficulty of getting reliable figures was very considerable. In the first place there are a good many ownerless cats that frequent a house or houses where they get food. There is some risk of counting some of these cats twice over. The second difficulty was due to the suspicious attitude of the people who seemed to imagine that we required all the information that we were seeking for some purpose connected with taxation. More than once we found them deliberately giving us false information. In spite of these difficulties we believe that the following figures possess a fair degree of accuracy.

It would appear that at the present time cats are very numerous in the Sadar Bazaar and comparatively scarce in the city. In the city, with a population of 23,885 there were only 314 cats. In the Sadar Bazaar with a population of 3500 there are 282 cats. In spite of this fact plague was, as we have shown above, more severe in the Sadar Bazaar than in the city, during the epidemic under report, and this we ascribed to the greater rat population in the Sadar Bazaar (vide p. 467).

In the city in 124 houses that possessed one or more cats the number of rats caught was 1187, an average of 9·5 rats per house. In 1799 houses that contained no cats 19,497 rats were caught, an average of 8·3 per house.

In the Sadar Bazaar in 119 houses that contained one or more cats, the number of rats caught was 902, an average of 7·5 rats per house. In 251 houses that contained no cats, 2175 rats were caught, an average of 8·6 rats per house.

These figures seem to show that the ordinary Indian cat is not a factor of any great value in keeping down the rat population.

Approaching the problem from a different standpoint, we found that 263 houses out of a total of 3813 inhabited houses kept cats. In these 263 houses there were 19 cases of plague, *i.e.* one in fourteen. In the 3550 houses that kept no cats there were 277 plague cases, an average of one in thirteen. The amount of protection that was offered by cats was therefore very small indeed.

TABLE V.

Census of Belgaum.

					City	Sadar Bazaar
Total number of houses	4927	793
Do. inhabited	3813	642
Do. uninhabited...	1114	151
Population	23885	3507
Number of houses in which rats were caught	2422	370
Number of rats	20684	3077
Average number of rats per house	8.5	8.3
Number of houses that contained buffaloes, bullocks, cows, ponies, or goats	1112	82
Number of such animals	3280	180
Number of houses containing such animals in which rats were caught	623	42
Number of rats	5738	402
Number of houses not containing such animals in which rats were caught	1799	328
Number of rats	14946	2675
Number of plague cases in houses that contained such animals	85	11
Number of plague cases in houses not containing such animals	211	56
Number of houses that contain cats	263	267
Number of cats	314	282
Number of houses containing cats in which rats were caught	124	119
Number of rats	1187	902
Number of houses not containing cats in which rats were caught	2298	251
Number of rats	19497	2175
Number of plague cases in houses that contain cats	19	21
Number of plague cases in houses that contain no cats	277	46

TABLE VI.

Type of house	Houses in which more than twenty but less than fifty rats were caught	Houses in which more than fifty but less than one hundred rats were caught	Houses in which more than one hundred rats were caught
Dwelling houses			
Brahmin	4	—	—
People in "service"	40	4	—
Coolies	33	1	—
Native Doctors	4	—	—
Pensioners	3	—	—
Agriculturists	34	4	—
Students	2	—	—
Pleaders	3	—	—
Beggars	1	—	—
Sweepers	1	—	—
Priest	1	—	—
Houses in which trades are carried on			
Sweetmeat shop	5	—	—
Tailors	8	—	—
Grocers	11	2	1 (197 rats)
Weavers	18	1	—
Grain merchants	7	1	—
Hotel keepers	3	—	—
Goldsmiths	5	—	—
Basket makers	6	—	—
Soda water shop	2	—	—
Farrier	2	—	—
Leather worker	3	—	—
Oil merchants	5	—	—
Carpenter	1	—	—
Potter	1	—	—
Barber	1	—	—
Fish dealer	1	—	—
Bone dealer	1	—	—
Wood merchant	1	—	—
Silk spinner	1	—	—
Tobacco seller	1	—	—
Betal nut seller	1	—	—
Vegetable shop	1	—	—
Liquor shop	1	2	—
Butcher	—	1	—
Stables	3	1	—
Unclassified	22	—	—
Totals	237	17	1

XXXVII. OBSERVATIONS ON PLAGUE IN POONA.

PART I.

Description of Poona City and Suburbs.

- A. Geographical position.
- B. General description of the city:—construction of houses, sanitation, people, communication by road and rail.
- C. Climate.
- D. Former epidemics.
- E. Plague preventive measures.

PART II.

Method adopted for studying the conditions prevailing in Poona and the data obtained.

- A. Plan of operations.
 - (1) Arrangements in connection with work in the city.
 - (2) Arrangements in connection with work in the laboratory.
- B. The plague epidemic and epizootic of 1908—1909.
 - (1) Plague in the suburban municipal area.
 - (2) Plague in Kirkee Cantonment.
 - (3) Plague in Poona Cantonment.
 - (4) Plague in Poona City.
- C. Data bearing on the factors which determine the seasonal prevalence of plague, collected in Poona 1908—1909.
 - (1) Climatic conditions.
 - (2) Variation in the virulence of the bacillus.
 - (3) Variation in the total number of rats.
 - (4) Variations in the proportion of immune to susceptible rats.
 - (5) Variation in the number of fleas found on the rats.

PART III.

Deductions drawn from the data collected, as to the seasonal prevalence of plague in Poona.

- A. The significance of imported infection.
- B. The influence of climate on plague in Poona.
- C. The influence of the virulence of the bacillus on plague in Poona.
- D. The influence of variation in the number of rats and in the proportion of immune to susceptible rats on plague in Poona.
- E. The influence of the variation in the number of fleas per rat on plague in Poona.

PART IV.

Conclusions.

PART I.

DESCRIPTION OF POONA CITY AND SUBURBS.

A. *Geographical Position.*

POONA, the most important city of the Deccan, is situated 75 miles S.E. of Bombay on the eastern watershed of the range of Western Ghats, 1850 feet above the sea level. It lies on the Basalt Rock, with a surface layer of Deccan Trap and Black-Cotton Soil.

The name generally includes (see Map I):—

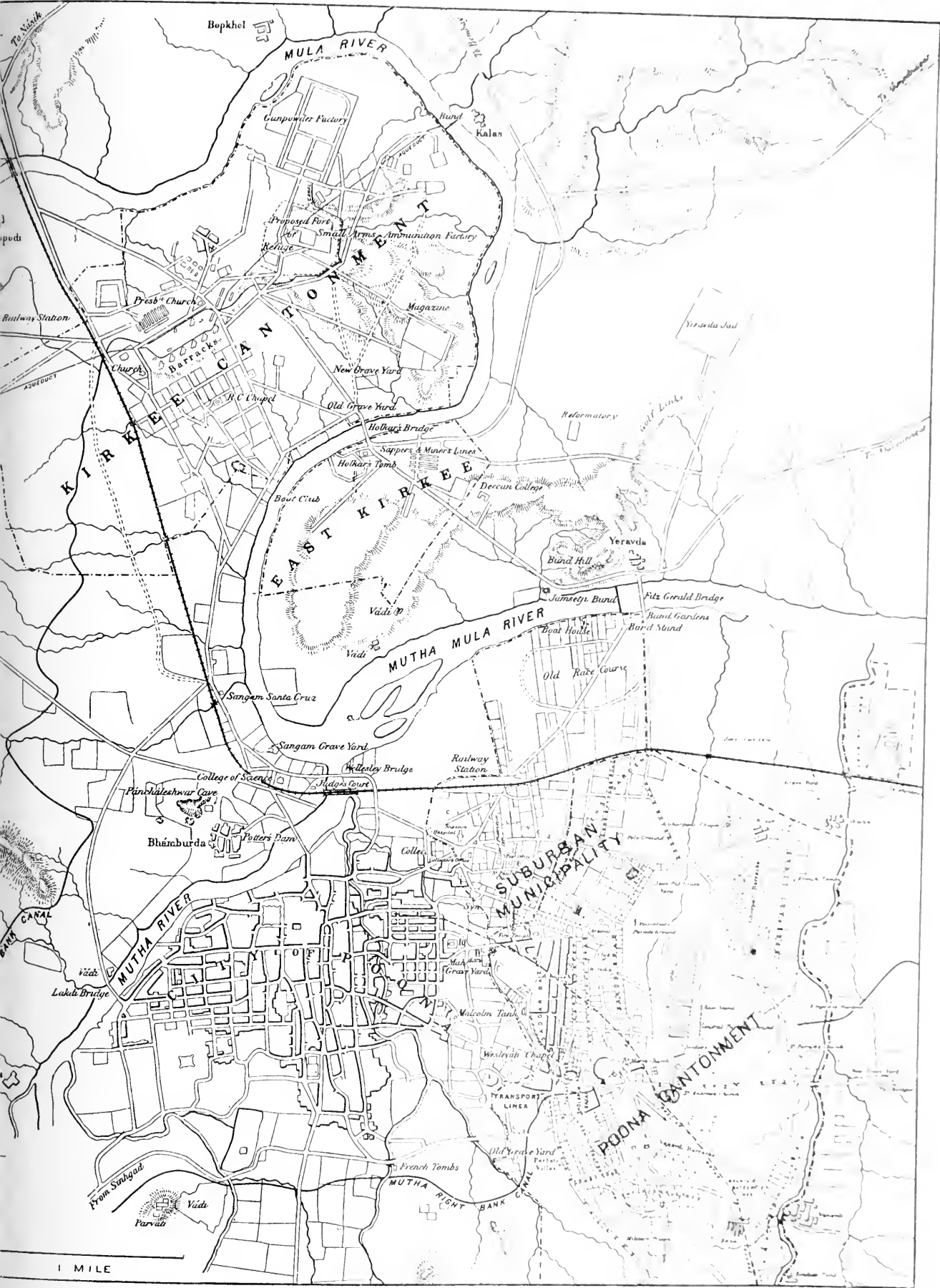
1. A large Native City.
2. The Poona Cantonment.
3. The Suburban Municipality.
4. The Cantonment of Kirkee.

These centres of population cover a very wide area so that at the outset of our observations we determined to limit our operations to the Native City only. Occasional reference has, however, to be made to the area surrounding the city, for extensive and frequent intercourse is maintained between the city and these suburban districts.

1. *The Native City.* This covers a roughly triangular area of about three square miles. The apex of this triangle is directed northwards and approximates, as will be seen on the accompanying map, to the union of the Mula and Mutha rivers (Map II).

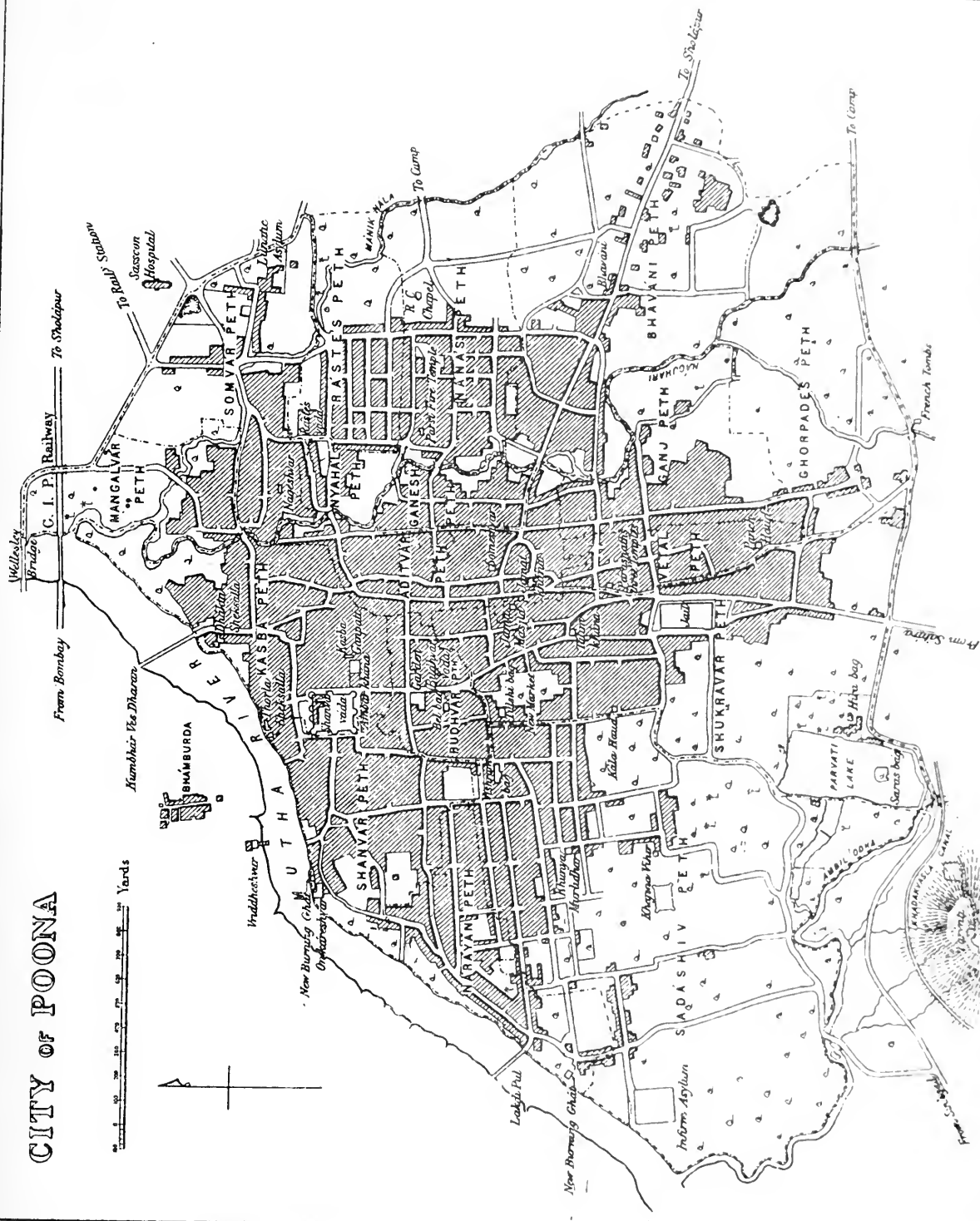
On the north-west the Mula with its tributary the Mutha river separates the city from the cantonment of Kirkee. Two small divisions of the city, however, namely the Peths of Bhamburda and Pulachiwadi, lie on the further bank of the river. These Peths are connected with the main portion of the city by three bridges and a stone causeway. On the north-east a comparatively sparsely populated tract of land separates the city from the Poona Cantonment and Suburban Municipality. On the south the city is bounded by extensive gardens and orchards. Population 111,381.

2 and 3. *The Poona Cantonment and the Suburban Municipality.* These areas lie along the north-eastern boundary of the city, the cantonment occupying a position to the south of the Suburban Municipal area. The bungalows of Europeans and wealthy natives are situated in the municipal area while the barracks of the European and native soldiers and the houses of the military officers and other European officials are situated in the cantonment. Here are stationed two



POONA

CITY of POONA



British infantry regiments, one native cavalry and three native infantry regiments with a transport corps. The Suddar or cantonment bazaar is situated between the barracks and the native city. This bazaar is made up chiefly of shops and the habitations of the natives who cater for the European and native population detailed above. In structure this bazaar resembles closely the city with which it is coterminous but the buildings and their surroundings are kept in better order, the sanitary arrangements being under the control of the military authorities.

The railway station is situated in the suburban area to the north-east of the city.

The population of the cantonment according to the census of 1901 was 32,777 and that of the suburban municipality 9162.

4. *Cantonment of Kirkee.* The cantonment of Kirkee, separated from the city by the Mutha and Mula rivers, lies to the north-west on the road to Bombay. Here are stationed three batteries of artillery, a company of sappers and miners and a native infantry regiment. There is also a large arsenal and ammunition factory. The factory employs a number of native hands. As in the Poona cantonment there is here also a Suddar bazaar which supplies the population of Kirkee with provisions and other necessities. This bazaar is distant about three miles from Poona City. The population of this cantonment is 5640.

The district around Poona. Poona City lies in a valley, the surrounding district therefore is more elevated. The country for some miles around the city is rocky and sparsely cultivated, except for a small portion which is irrigated by means of a canal. There are therefore few villages of importance in the immediate neighbourhood of the city.

B. *General description of city—construction of houses, sanitation etc.*

The central portion of the city is the oldest and most densely populated part. The houses are here ill constructed, crowded together, with few open spaces around them. The houses on the east and west of the city are better built, while on the south the buildings are more scattered and are surrounded by large gardens and orchards.

The streets of the city are, for the most part, fairly wide. Compared with the average Indian city there are only a few alleys so that the majority of houses open on to the main streets.

A large market is situated to the south of the centre of the city. Fruit, vegetables and other provisions are brought here daily for sale. Surrounding the market are a number of shops which retail grain, groceries, and stores to the villagers, and others who come to the market for their provisions. The chief grain stores, however, are situated in Nana's Peth in the eastern part of the city. To this wholesale grain market large quantities of grain are brought chiefly from the Ahmednagar and Thana districts but some also from the surrounding country.

It is impossible to describe the houses of the city under any one type as they vary so greatly in size, shape, and construction. One type of house very commonly seen in Poona, however, is built in the form of a square with a court-yard of varying size in the centre. This court-yard or quadrangle is usually well kept, clean and covered with flag-stones or used as a small garden. It may contain a well, but usually the water supply is laid on by pipes. One corner of this quadrangle is often set apart for cattle, but in many cases these animals occupy one of the rooms looking out on it. As a rule half the area of the ground floor is practically an open verandah. The front part of the building is reserved for latrines, stables and stores. Small booths open into the part of the building which faces the street. These booths are like cupboards (often no larger) and are sublet to tradespeople who spend part of the day there, locking up their shops at night; sometimes however they sleep on the premises.

The living rooms are on the ground floor. In some cases these are so dark that food has to be partaken of by the light of a lamp. The sleeping apartments are upstairs (these houses are usually of two stories) and between the apartments and the roof there may be a loft, used as a store-room for grain and much that is apparently rubbish. The contents of these rooms are seldom cleaned out or disturbed. The houses are generally built on a plinth two or three feet high which is faced with stone; the floors are of beaten-down earth covered with a layer of cowdung spread on when moist. The walls are usually made of bricks which are baked, but sometimes only sundried. The roof is covered with two or more layers of tiles upon a framework of bamboo battens. The tiles may be cylindrical, like the Bombay variety, but are usually flat, rough, and spade shaped. Rarely the roofs are made of corrugated iron, or mangalore tiles. The windows are not generally filled in with glass, but can be closed with wooden shutters, which are left open during the day to admit light, and thus, at this time, the houses are well ventilated.



Typical road in Poona showing the peculiar roof.



Grain shop in Poona.



Homes of this description belong generally to the middle class and are probably the commonest type of house in Poona.

The houses of the poorer classes are small, irregularly built, and badly designed; their floors and walls are made of mud with a roof of rough flat Deccan tiles. They are single storied buildings which admit very little light and air through the small holes in the walls which serve the purpose of windows. Nevertheless such houses, especially when occupied by Hindus, are often kept very clean, the floor being frequently plastered with fresh cowdung.

But the houses of the poor are not by any means always ill-ventilated or badly lighted. In some parts, especially in the south of the city, houses occupied by weavers are met with, which consist chiefly of one large room, and this serves alike the purpose of a dwelling room and workshop. This room has no loft, and is so constructed as to admit freely light and air on all sides. Such houses, occupied by the poor, leave little to be desired from a sanitary point of view.

The poorest and humblest class of people, the Mangs, Mahars, sweepers and such like, live in rude huts covered with pieces of tin and rags. Colonies of these people dwell in the north of the city in Mang-alwar Peth, in the east in Nana's Peth, and also in Sadashiv and Bhamburda Peths.

The city for municipal purposes has been partitioned into a number of irregular divisions called Peths (literally markets). There are nineteen of these Peths which contain separately from twenty to fifteen hundred houses. In each Peth the houses are numbered consecutively so that generally it is easy to locate a house by its number. In some instances however the numbers on the houses are irregularly distributed with wide intervals between two houses with consecutive numbers. In some cases, too, houses which once existed and were numbered have fallen into a state of ruin or have entirely disappeared. Other houses again are without number plates so that their number had to be guessed by the position they occupied in relation to the adjoining and properly numbered houses. A single number is occasionally applied to a whole group of dwellings; a group of such houses probably arose around a single house at a date subsequent to the time when the numbers were originally given to the houses. These facts have been mentioned in order that the reader may understand some of the difficulties met with in carrying out the observations in Poona City, difficulties which, unfortunately, make some of the records we have compiled not quite as accurate as they might be; any error however from this cause can

generally be overlooked except where attention has particularly been drawn to the matter. The area, population, and number of houses in each Peth are given in Table I.

Sanitation. The sanitary arrangements of the city are very primitive. Latrines are cleaned out by sweepers and their contents removed

TABLE I.

Population of Poona City.

Table showing the population, and the area in square yards, of the different Peths of Poona City, also the castes preponderating in each Peth according to the census of 1901.

Serial No.	Name of Peth	No. of houses	Area in sq. yds	Population	No. of inmates per house	Density per 1000 sq. yds	Castes (chiefly)
*1	Shukurawar	1,638	955,000	14,707	9.0	15	Almost all castes.
2	Kasba	1,510	575,000	12,965	8.6	23	Poor working class.
3	Rawiwar or Aditwar	1,435	325,000	9,254	6.5	28	Jains & Merchants.
*4	Sadashiv	823	2,275,000	8,959	10.9	4	Brahmins.
*5	Bhawani	1,072	1,235,000	8,451	7.9	7	Mahomedans & Merchants.
6	Shanwar	608	445,000	8,140	13.4	18	Brahmins.
*7	Nana	772	525,000	7,069	9.2	13	Mangs & Mahars.
8	Budhwar	545	185,000	5,951	10.9	32	All castes.
*9	Gunj	864	428,000	4,894	5.7	11	Jains & Kumbhars.
*10	Somwar	417	545,000	4,629	11.1	8	Gosavee & others.
11	Vetal	664	195,000	4,625	7.0	24	Gujratee & others.
12	Ganesh	457	155,000	4,168	9.1	27	Poor working class.
*13	Rasta	512	365,000	4,122	8.1	11	Madrasi & others.
14	Narayan	486	375,000	3,997	8.2	11	Brahmins & Marathas.
*15	Bhamburda	500	2,906,220	3,645	7.3	1	Marathas.
*16	Mangalwar	351	485,000	2,904	8.3	6	Mahar & Sweeper.
17	Nyhal	101	105,000	1,243	12.3	12	Rich people.
*18	Ghorpadi	302	655,000	1,230	4.1	2	Mahomedans & Shoe makers.
*19	Gultekdi	21	2,447,300	428	?	0.17	Chiefly Marathas.
*20	Pulachiwadi	156	Part of Bhamburda. Included in Bhamburda.				
Total			15,181,520	111,381		7.3	

Note.—In those marked with a star the density of population is misleading; being situated on the outskirts of the city a large part of each peth consists of open fields and orchards, the remaining part being often very densely populated.

It will be noticed that the part most densely populated is in the centre of the city, viz. Budhwar, Rawiwar, Ganesh, Vetal and Kasba.

by means of carts. Waste water, running into gutters, is sometimes used to irrigate a small garden, often it is allowed to stagnate and vaporate in the sun. A large under-ground drain runs down one or two of the main thoroughfares and into it a certain amount of sullage and storm water finds its way. The storm water as a rule is carried away in open gutters by the side of the road. In parts of the city, where the traffic is greatest, these roadside gutters are covered over with flat slabs of stone.

There are many wells scattered throughout the city. They are used chiefly for washing purposes, for a good supply of water is laid on in pipes to almost every house.

People. The population of Poona City according to the census of 1901 was 111,381, but it is probable that owing to the ravages of plague the population at the present time is about 100,000.

The following are the different castes amongst the people of Poona City according to the census of 1901 :—

Hindus	97,298
Mahomedans	11,332
Jains	1,133
Christians	952
Jews	483
Parsis	168
Others	15
Total	111,381

The people inhabiting the city are generally poor. Their industries and trades are few in number and of little importance; namely: brass work, silk and cotton weaving, the manufacture of gold and silver threads, and of glass bangles. Of recent years, paper, sugar and pinning factories have been started on modern lines: these, as well as the government small-arm ammunition factory at Kirkee, employ a considerable amount of native labour. These works are all situated outside the city, although most of the workmen live within its boundaries.

Communication by road and rail. Poona lies on the main line of the Great Indian Peninsular Railway between Bombay and Madras. A branch railway also leaves the main line here for Belgaum and the Southern Maratha country. Many excellent roads round Poona afford communication with every part of the surrounding district. Large quantities of agricultural produce are brought for sale to the city daily by road and rail.

C. Climate.

Owing to its elevation and comparative proximity to the sea-coast, the climate of Poona is temperate for most of the year. For purposes of description the year may be divided into four equal seasons.

(1) June, July and August: the rains proper.

(2) September, October and November: a period succeeding the rains.

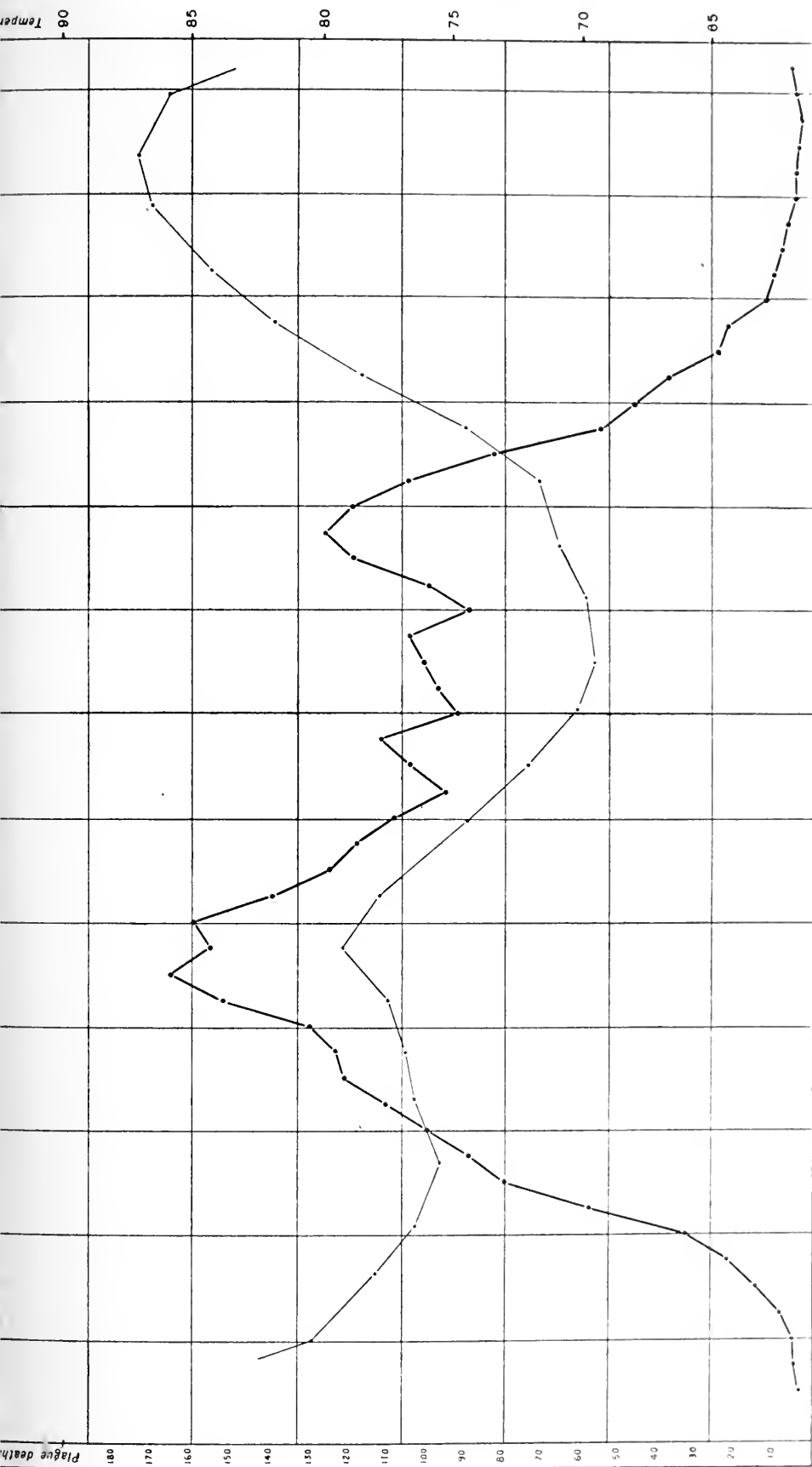
(3) December, January and February: the cold weather.

(4) March, April and May: the hot weather.

1. The rains:—During this period by far the largest part of the total rainfall is precipitated. The rain begins to fall early in June, at first in the form of showers with considerable intervals of sunshine and clear weather, but during July and August rain falls almost daily. Towards the end of August the interval between each precipitation again lengthens, and a few clear rainless days occur at this time. The temperature in the beginning of June, with a mean of over 80° F. and a diurnal variation of about fifteen degrees, rapidly falls through June and July to a mean slightly over 75° F., the diurnal variation meanwhile being reduced to less than ten degrees. The atmospheric humidity in the beginning of June rises rapidly from a mean of about 60 % to over 80 % in July and August.

2. The period after the rains:—During the latter part of September a few showers of rain fall; the intervals between the showers become more and more prolonged till the month of October is practically free from rain. The temperature which had fallen with the development of the rains in July and August begins to rise again in September to a mean of nearly 80° F., but at the end of October, the temperature again falls rapidly to a mean of about 70° F. in the end of November. The diurnal variation in the temperature during this period also gradually increases from approximately ten degrees in the beginning of September to as many as thirty-five degrees in the end of November. The humidity during this period falls rapidly from 80 % in September to about 60 % at the end of November.

3. The cold weather:—As a rule very little if any rain falls during this season of the year, but an occasional shower may occur especially in January. The climate at this time of the year is delightfully cool, the mean daily temperature seldom rises above 75° F. except



POONA

— Average weekly number of plague deaths in Poona City 1897—1909
- - - Average half-monthly temperature 1897—1906

for a few days towards the end of February. The diurnal variation in the temperature remains considerable, a difference of 35 degrees being often noticed between the maximum and minimum readings. The humidity at this time of the year remains fairly constant between 50 and 60 %, but falls slightly towards the end of February when 45 % is often registered.

TABLE II.

Table showing the average half-monthly mean temperature for 10 years, 1897—1906 inclusive.

Half-monthly periods		Average
January	1	69·9
January	2	70·8
February	1	71·6
February	2	74·5
March	1	78·6
March	2	81·8
April	1	84·3
April	2	86·3
May	1	87·0
May	2	85·8
June	1	83·6
June	2	80·6
July	1	77·9
July	2	76·5
August	1	75·7
August	2	76·3
September	1	76·6
September	2	77·3
October	1	79·5
October	2	77·7
November	1	74·6
November	2	72·1
December	1	70·2
December	2	69·5

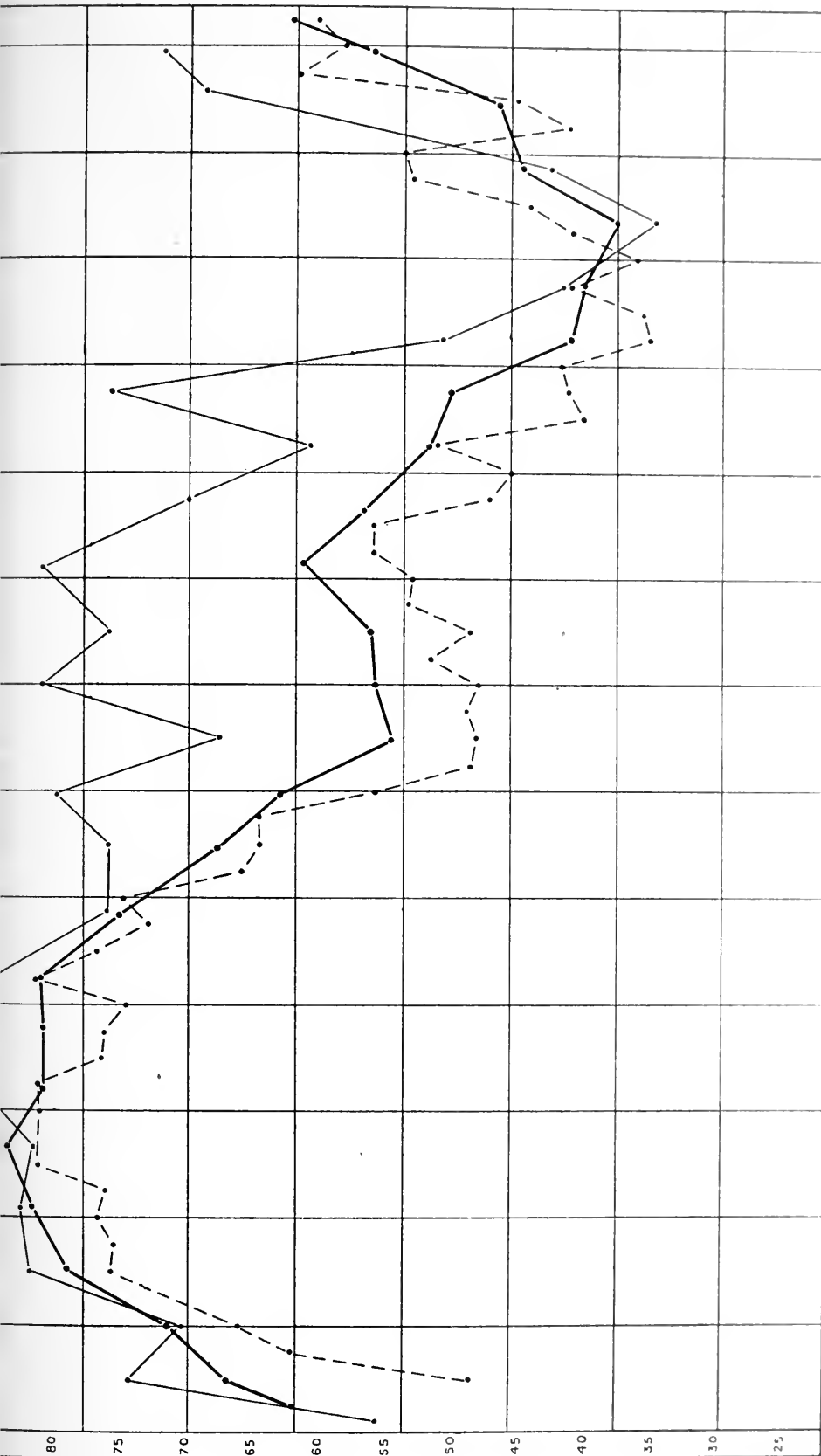
TABLE III.

Average humidity in half-monthly periods for 10 years from 1897 to 1906.

Half-monthly periods		Average
January	1	61·4
January	2	57·5
February	1	51·9
February	2	50·5
March	1	41·4
March	2	40·7
April	1	38·0
April	2	45·2
May	1	47·0
May	2	56·4
June	1	67·2
June	2	71·8
July	1	79·5
July	2	83·4
August	1	83·8
August	2	81·1
September	1	81·0
September	2	81·1
October	1	73·3
October	2	67·7
November	1	63·3
November	2	54·7
December	1	56·4
December	2	56·5

The figures represent the percentage of saturation.

4. The hot weather :—This period is almost always rainless but an occasional shower may occur in May. The temperature, which had already begun to rise in the end of February, continues to rise rapidly till a mean daily temperature of 87° F. or higher is registered in the beginning of May. The diurnal variation also still remains considerable,



POONA

- Average humidity in Poona for a period of 10 years 1896—1907
- - - Average humidity in Poona for the years 1908—1909
- Average humidity in Poona for the years 1902—1903. 5th epidemic

CHART III

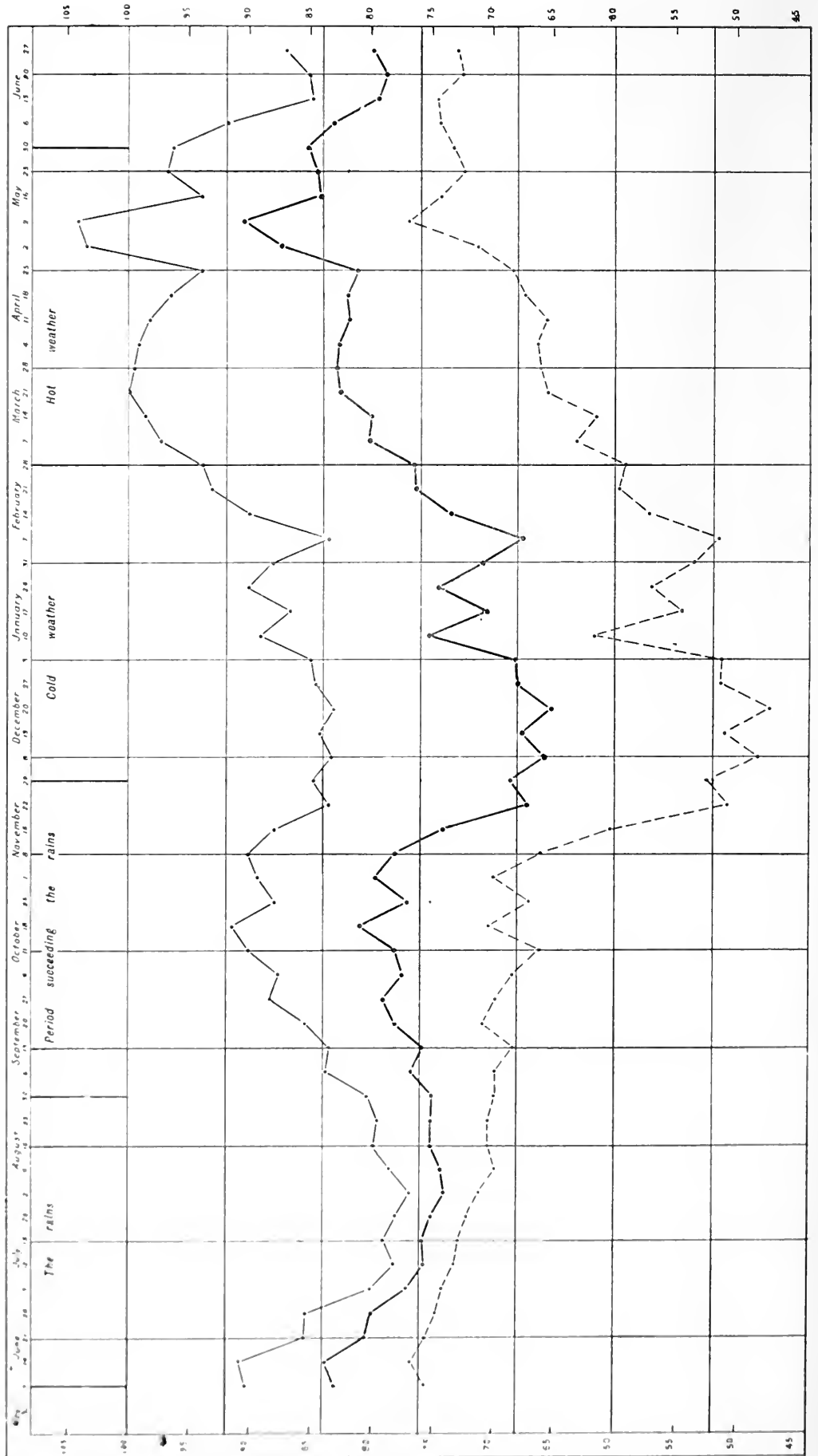


TABLE IV.

Table showing the number of plague deaths in Poona City in periods of seven days for the years 1897 to 1907 inclusive.

Week ending	1897	1898	1899	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	Total
Jan. 3	—	41	2	0	16	288	283	142	239	Nil	Nil	42	21	1074
10	—	99	0	0	3	340	357	169	176	1	2	16	26	1189
17	—	77	0	1	1	304	630	205	155	1	0	33	21	1428
24	—	57	0	1	3	292	787	171	149	0	1	27	27	1515
31	—	33	1	4	2	220	871	147	114	2	0	17	17	1428
Feb. 7	—	37	1	0	2	172	818	130	92	0	0	0	9	1261
14	—	26	4	1	2	118	595	132	93	4	0	0	2	977
21	—	8	17	0	2	76	456	76	47	9	0	0	6	697
28	—	12	43	0	1	59	301	95	38	9	1	0	4	563
Mar. 7	—	2	71	2	3	38	208	89	40	4	0	1	0	458
14	—	3	64	0	1	14	107	81	20	4	1	0	0	295
21	—	3	90	0	0	8	78	65	18	4	0	3	2	271
28	—	2	89	1	0	3	23	28	7	6	1	0	0	160
April 4	—	2	74	0	0	3	15	30	9	4	1	0	1	139
11	—	2	74	1	0	1	10	5	7	2	0	0	1	103
18	—	1	74	1	1	0	8	1	1	5	0	0	0	92
25	—	0	60	0	1	0	1	2	1	3	0	0	0	68
May 2	—	2	64	0	0	0	0	0	2	2	0	0	1	71
9	—	0	60	1	0	0	0	0	0	0	0	0	1	62
16	—	0	41	0	1	0	0	0	0	2	0	0	0	44
23	—	1	57	0	1	0	0	0	0	0	0	0	0	59
30	—	0	71	0	2	0	0	0	0	1	0	0	0	74
June 7	7	0	44	0	0	0	0	0	0	1	0	0	0	52
14	7	0	56	0	0	0	0	0	0	0	0	0	0	63
21	7	0	56	0	0	0	0	0	0	1	0	0	0	64
28	3	1	94	0	0	1	0	0	0	0	0	0	0	99
July 5	5	0	181	0	0	0	0	0	0	1	0	1	0	188
12	12	0	253	0	0	0	0	0	0	3	0	0	—	268
19	5	0	372	0	0	0	0	0	0	19	0	0	—	396
26	0	0	651	1	0	0	0	0	0	48	0	0	—	700
Aug. 2	2	0	886	5	0	1	0	4	1	64	0	3	—	966
9	7	0	894	18	0	0	0	1	0	140	0	2	—	1062
16	8	0	908	25	0	0	0	3	0	245	1	4	—	1194
23	3	0	985	28	1	1	1	2	0	303	1	0	—	1325
30	8	2	992	62	0	1	0	2	0	394	0	1	—	1462
Sept. 6	10	2	813	75	1	4	0	3	0	556	7	15	—	1486
13	18	0	598	161	1	13	6	3	0	736	11	20	—	1567
20	38	0	495	325	2	6	6	8	4	897	26	26	—	1833
27	62	0	348	421	7	7	7	32	1	1013	35	50	—	1983
Oct. 4	75	0	250	541	9	3	5	69	2	813	63	38	—	1868
11	108	1	183	583	18	11	24	100	1	726	71	89	—	1915
18	185	0	84	543	18	9	26	111	2	465	84	144	—	1671
25	263	0	60	398	31	1	51	173	0	281	102	137	—	1497
Nov. 1	359	0	24	337	41	11	80	180	1	168	126	96	—	1423
8	326	0	19	231	80	22	82	270	0	71	96	102	—	1299
15	287	0	6	139	85	25	121	241	0	62	87	92	—	1145
22	336	0	4	134	117	30	108	306	1	27	94	91	—	1248
29	343	1	2	89	212	65	116	361	0	11	79	62	—	1341
Dec. 6	266	0	1	50	157	65	123	309	0	10	65	49	—	1104
13	229	1	2	35	219	89	172	307	0	2	65	50	—	1171
20	230	0	0	39	227	180	122	321	2	0	47	42	—	1210
27	164	0	0	38	269	306	141	273	0	0	39	42	—	1272
Total	3373	416	10218	4291	1537	2787	6739	4647	1223	7120	1106	1295	139	41781

the maximum and minimum temperatures differing from one another in from 25 to 30 degrees. The humidity during this period reaches a minimum in April of approximately 35 %. The moisture in the air however increases with the onset of the S. W. monsoon breezes so that at the end of May as much as 60 % or more of moisture may be recorded.

These variations in the climatic features of Poona can be readily followed in Charts I, II and III as well as in Tables II and III.

D. *Former Epidemics* (Chart VIII).

From the time plague first appeared in Poona City some eleven years before the commencement of the present observations the disease has continued to prevail to some extent at least every year. The disease has occurred generally in well marked epidemics, but between each epidemic sporadic cases have been registered. Those persons who

TABLE V.

Serial no. of epidemic	Epidemic began in week ending	Epidemic ended	Maximum attained week ending	Total deaths from plague during epidemic
First	Probably Feb. or Mar. accurate figs. not available	30th April 1898	1st Nov. 1897	3778*
Second	31st Jan. 1899	13th Dec. 1899	30th Aug. 1899	10216
Third	26th July 1900	14th Mar. 1901	11th Oct. 1900	4314
Fourth	1st Sept. 1901	11th April 1902	10th Jan. 1902	3430
Fifth	23rd Aug. 1902	25th April 1903	31st Jan. 1903	6496
Sixth	13th Sept. 1903	25th April 1904	17th Jan. 1904	2758
Seventh	2nd Aug. 1904	2nd May 1905	29th Nov. 1904	4287
Eighth	10th Jan. 1906	18th Dec. 1906	27th Sept. 1906	7120
Ninth	1st Sept. 1907	31st Jan. 1908	1st Nov. 1907	1232
Tenth	30th Aug. 1908	28th Feb. 1909	18th Oct. 1908	1289

* The figures for the first epidemic are not complete, accurate figures for the City are available from June only.

have died between the epidemic periods, we have reason to believe, obtained their infection outside the city, many of them in Bombay. This is a matter not to be wondered at when it is borne in mind that Poona is within easy reach of Bombay by a four hour railway journey, and that the epidemic period in Bombay coincides almost exactly with the healthy period in Poona. Moreover, except during the early epidemics, no restrictions have been placed on persons coming from infected houses. Apart from these imported cases of plague, there have

been ten well marked epidemics of the disease in Poona City during the past twelve years. The severity of these epidemics has varied considerably, and they have occurred at different periods of the year as will be seen from the Tables IV and V and from Chart VIII. The explanation we have to offer for the variation in the intensity of the epidemics will be postponed till we have detailed our experience of the tenth epidemic which we have been enabled to study in detail.

E. *Preventive Measures.*

A few remarks remain to be made regarding the measures which have been carried out to prevent the spread of the disease in past years. Till the year 1907 the chief measures adopted had been the disinfection of infected houses with perchloride of mercury; evacuation of infected houses when they were not disinfected; isolation of the sick in hospital when possible. Inoculation with anti-plague vaccine had been resorted to, but few people availed themselves of this measure.

In 1907 Government appointed a committee of influential Indian gentlemen and placed in their hands funds to be used for measures against plague. This enlightened committee carried out an extensive scheme of operations which resolved itself in instructing the people by holding public meetings and circulating popular pamphlets on the most recent views of the manner in which plague is spread, and urging and encouraging the people by pecuniary assistance and otherwise (*a*) to resort to evacuation of their houses as soon as rats were observed to be dying, and before cases of the disease occurred among the household, (*b*) to be inoculated with anti-plague vaccine if evacuation was not possible, (*c*) to destroy rats.

As a result of these measures, during the past two years there has been a considerable decrease in the number of plague deaths. The extent to which these measures were adopted can be gauged from the following figures. The municipal authorities estimate that during the epidemic of 1908—09 four thousand nine hundred persons occupied municipal huts in the plague camps, and that five thousand persons lived in private huts in the fields; they also estimate that between fifteen and twenty thousand persons left the city for the surrounding towns and villages. By this rough estimate it would appear that approximately one quarter of the population of Poona City evacuated their houses during the recent epidemic. Records showing the numbers who were inoculated with anti-plague vaccine from year to year are

much more accurate. The following figures were obtained from the municipal authorities:—

Year	Numbers inoculated
1901—02	92
1902—03	384
1903—04	716
1904—05	468
1905—06	516
1906—07	6,000
1907—08	11,593
1908—09	16,998

PART II.

METHOD ADOPTED FOR STUDYING THE CONDITIONS PREVAILING IN POONA AND THE DATA OBTAINED.

A. *Plan of operations.*

As has been detailed in the introduction to this report, one of the main objects of this enquiry was to determine whether the factors, which influence the seasonal prevalence of plague and which were put forward in one of our previous reports (*Journ. of Hygiene*, vol. VIII. No. 2, p. 273), after a study of the facts elicited from our experiments and observations in Bombay and in the Punjab, held good for Poona, which city, although so adjacent to Bombay (75 miles), presented an entirely different seasonal prevalence of the disease. In conducting this enquiry therefore we had to make arrangements to determine :

- I. The onset, course, and termination of the epizootic and epidemic.
- II. To collect data bearing on each of the factors which we believed had an influence in determining the seasonal prevalence of plague, *i.e.*
 - (a) climatic conditions,
 - (b) variations in the virulence of the bacillus,
 - (c) variations in the total number of rats and variations in the proportion of immune to susceptible rats,
 - (d) variations in the number of fleas.

To this end we made arrangements with the municipal authorities to assist us in collecting daily a fair sample of the rats of the city, and to furnish us with information, as early as possible, regarding any plague cases or deaths that came to the knowledge of their Health Officer. At the same time we improvised a temporary laboratory in a convenient position where the rats which were collected could be examined,

and the various data mentioned above could be registered. The details of these arrangements can most conveniently be considered under the heads

- (1) Arrangements in connection with work in the City.
- (2) Arrangements in connection with work in the laboratory.

(1) *Work in the city.*

- (a) *To obtain a good daily sample of rats.*
- (b) *To obtain information regarding the onset of the epidemic or epizootic.*

(a) *To obtain a good daily sample of rats.* A campaign against rats had been in force in the city during recent years. The rat catching staff was under the control of the Health Officer of the Municipality and consisted in a "Rat Inspector" and ten coolies. These men were provided with rat traps which they set in no systematic manner nor were the rats which were caught submitted to post mortem examination before being destroyed. By agreement with the municipal authorities this scheme was expanded and modified and the services of the rat catching staff were handed over to the Commission. Three additional "Rat Inspectors" were engaged by the Commission together with twenty-two coolies.

It was considered desirable to obtain from 150 to 200 rats daily, sufficient, on the one hand, to obtain accurate weekly statistics of the flea prevalence and breeding season of rats, and of the presence and the extent of an epizootic amongst them, and not too many, on the other hand, to interfere appreciably with the actual rat infestation of the city, nor with the careful and thorough examination of those caught throughout the year.

In these circumstances it was important that an even sample of rats should be obtained daily from every part of the city, and to attain this object the city was divided up by us into four wards, each having approximately an equal number of houses. Each ward was handed over to an inspector with a certain number of coolies and traps, and in each ward a room was hired to serve as a *depôt* in which the property in charge of the inspector was kept and where the traps were checked, washed, and oiled once a week.

Each of these wards was subsequently subdivided into two sections, making eight in all. These were then put in charge of eight *muc-cadums* or overseers who could read and write, and the inspectors were

reduced to two, whose duty it was to keep these muccadums at their work, and to see that it was done accurately.

About 100 traps were set in each of the eight sections. The houses were visited consecutively every afternoon, the traps having been previously baited with a small piece of ordinary bread dipped in sweet-oil.

The following morning these traps were all collected, and those containing rats were at once put into flea-proof canvas bags and conveyed to the laboratory, each cage having attached to it a label showing the number of rats and the address where they were caught, etc. This being written in vernacular on the back of the card, was subsequently translated into English by a clerk at the laboratory.

Every effort was made to induce the people to send dead rats to the laboratory for examination. Advertisements were inserted in vernacular papers, and the municipal authorities exerted their influence on the people. Rewards—increased at one time to as much as eight annas per rat early in the epidemic—were offered for dead rats if proved afterwards to be plague infected. Nevertheless dead rats were obtained only at very irregular intervals and not systematically from every part of the city; yet we were aware from our own experience in the city that from time to time during the epizootic large numbers of them were dying. At one time indeed it would scarcely have been possible to pass once through the city without seeing one or more lying dead in some gutter or dust-bin at the side of the road.

It was necessary that we should have some means for estimating as accurately as possible the rat infestation of the houses through the period of our observations. The only method that suggested itself to us to attain this end was to systematically trap the houses and estimate their rat infestation by recording the number of rats caught for every one hundred traps set. This method has of course many obvious fallacies; every effort was therefore made, from the outset, to keep this record as carefully as possible and to exclude every avoidable source of error; for example the work of the coolies in the city was frequently inspected and checked; the same kind of baits was always used in the traps, and heavy penalties were imposed on the coolies for setting traps without proper bait or using them when they were unserviceable or allowed the rats which were trapped to escape. Moreover, a most important precaution, as we were able to demonstrate later, was also adopted, namely, the use of one particular type of trap. We were able to show that with very slight modifications in the type of trap used

very different estimates of the rat infestation of houses as calculated by this method could be obtained; these variations depended apparently on the efficiency or otherwise of the trap used in each case. From Table VI it will be seen that our so-called "Commission" trap was more than six times as efficient as a type of trap we obtained from England and three times as efficient as a very similar trap which had a spring trap door in place of a weighted and balanced one.

TABLE VI.

Table showing the comparative value of different kinds of traps, set uniformly distributed in Poona City.

Period between which traps set	Kind of trap	Number of traps set	Number of rats caught	Number of rats per 100 traps set
Sept. 1 to Dec. 13 '08	Commission	43753	15623	35.7
Do. do.	English	13222	700	5.3
Dec. 14 to Mar. 14 '09	Commission	30386	6582	21.6
Do. do.	Spring flap	16051	1132	7.1
Aug. 15 to Sept. 19 '09	Commission	12399	4498	36.3
Do. do.	Tophole	1621	326	20.1

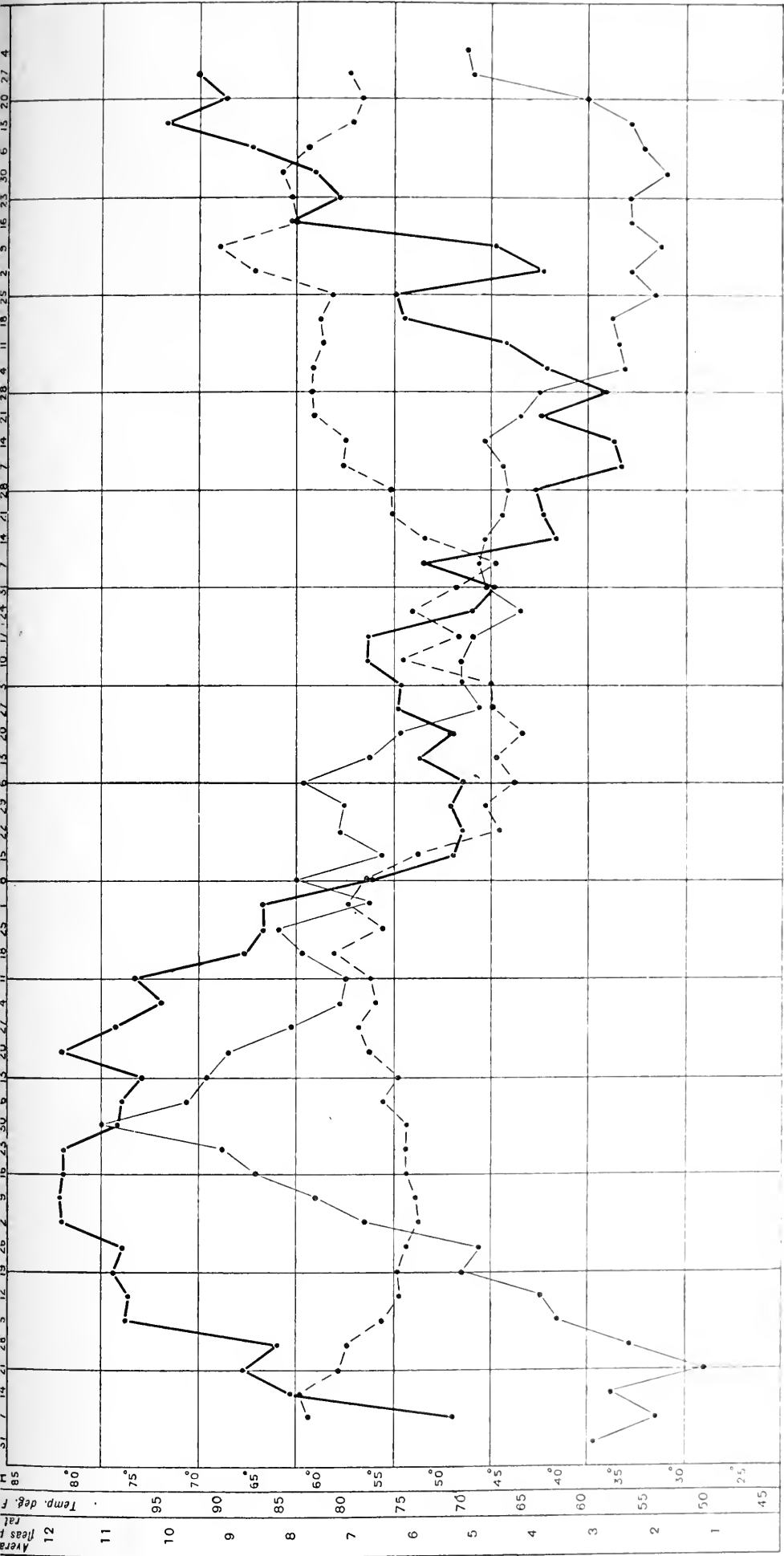
(b) *To obtain early information regarding the onset of the epidemic or epizootic.* In order to obtain the earliest possible information of plague we addressed the private practitioners of the city asking them to inform us at once of all suspected cases of the disease and to acquaint us of any rat mortality that came to their notice. The Health Officer was also good enough to forward to us any information of this nature he might acquire either indirectly or in his official capacity. It may be well here to give a brief description of the system of registration of deaths, which, as in most other Indian cities, is somewhat unreliable, and the precautions we took to remedy these defects as far as possible in compiling our figures. In Poona no corpse can be removed for burial or cremation without a pass which is supplied by the city Health Office to the friends or relations of the deceased. Opportunity is taken by the Health authorities when issuing this pass to obtain information regarding the cause of death. In a few instances only the patient has been attended during his last illness by a qualified medical practitioner. In the majority of instances, especially during the prevalence of an epidemic, the friends of the deceased merely detail a few of the main symptoms from which the patient has suffered and the duration of his disease to the health authorities who, on this information, arrive

at a diagnosis, and register the case accordingly. All the earlier cases of plague therefore were visited by us to obtain more accurate information and to ascertain any possible source of infection by inquiring into the habits and recent movements of the patient or his friends, and the presence or otherwise of other suspicious cases in the neighbourhood. Inquiry was also made as to whether dead rats had been observed and the opportunity was taken of offering a substantial reward for any that might be found subsequently and sent to the laboratory for examination. These measures were all the more essential in that the notification of plague cases is not compulsory in Poona City. These methods were carefully carried out in investigating the majority of the first hundred cases of plague, the few exceptions being those cases which could not be located or which occurred in an area already known to be infected. Twenty or thirty rat traps were set for a period of a week or ten days in the houses¹ in any suspected locality with a view to detecting plague among these rodents; any rats caught were kept for a few days so that the infected ones might fully develop the disease before being killed and examined. When possible also, *i.e.* when the room could be closed up so as to prevent the access of cats and dogs, or when the room had not been cleaned out, a guinea-pig was allowed to run about the room to act as a flea trap. Generally, however, information regarding plague cases was not received early enough to ensure success for this method, for the patient was often dead before information reached us or the house had been cleared out and evacuated. In some cases a guinea-pig could only be left in a room for an hour or two because the access of dogs and cats could not be prevented. For these reasons therefore our endeavours to obtain evidence of local infection by this method were not very successful.

(2) *Work in the laboratory.*

The work in the laboratory was conducted much on the lines already described in our previous reports. The rat traps, containing rats enclosed within a canvas bag, to which a label was attached giving particulars as to where the rats had been caught, were collected daily at the laboratory. Records were kept here of all houses in which traps were set; the number of traps set daily was also noted, as well as the number of rats caught. Each trap in turn together with its bag was placed in an air tight tin box which was furnished with a shallow tray

¹ These traps were not included in calculating the number of rats per 100 traps set.



POONA

- The average weekly humidity (6 hourly readings) Poona City 1908—1909
- - - The average weekly temperature in degrees F. 1908—1909
- The average number of fleas per rat 1908—1909

to facilitate the removal and enumeration of the rats and their fleas. Chloroform¹ was poured into the box which was then closed. By this means the rats and their fleas were killed. The total number of fleas found on the rats was recorded. The rats were then weighed, a card being attached to each giving particulars as to where it was caught and its weight. Each rat was then pinned out on a board for dissection: thereafter details were added on the card as to sex, pregnancy, number of foetuses and any pathological features observed. Any rat which presented the least suspicion of plague infection, either in an acute or chronic form, was subsequently submitted to a more detailed examination with a view to substantiating or refuting these suspicions. The cards having been collected at the close of the day's work, the matter written on these was entered into a register. Daily, weekly and monthly summaries of the work were compiled from this register; these summaries materially facilitated the labour involved in studying the large mass of facts collected in this way.

A certain number of dead rats were brought to the laboratory where they were examined, the result of this examination being also recorded. An experiment was also started to test, if possible, the extent to which immunity was developed among the rats during the course of the epizootic. The details of this experiment are recorded in a separate paper; incidentally it may be remarked that, although the experiment led to no definite conclusion as to the progressive development of immunity among the rats with the advance of the epizootic, yet it showed at least that some local strains of the plague bacillus were highly virulent.

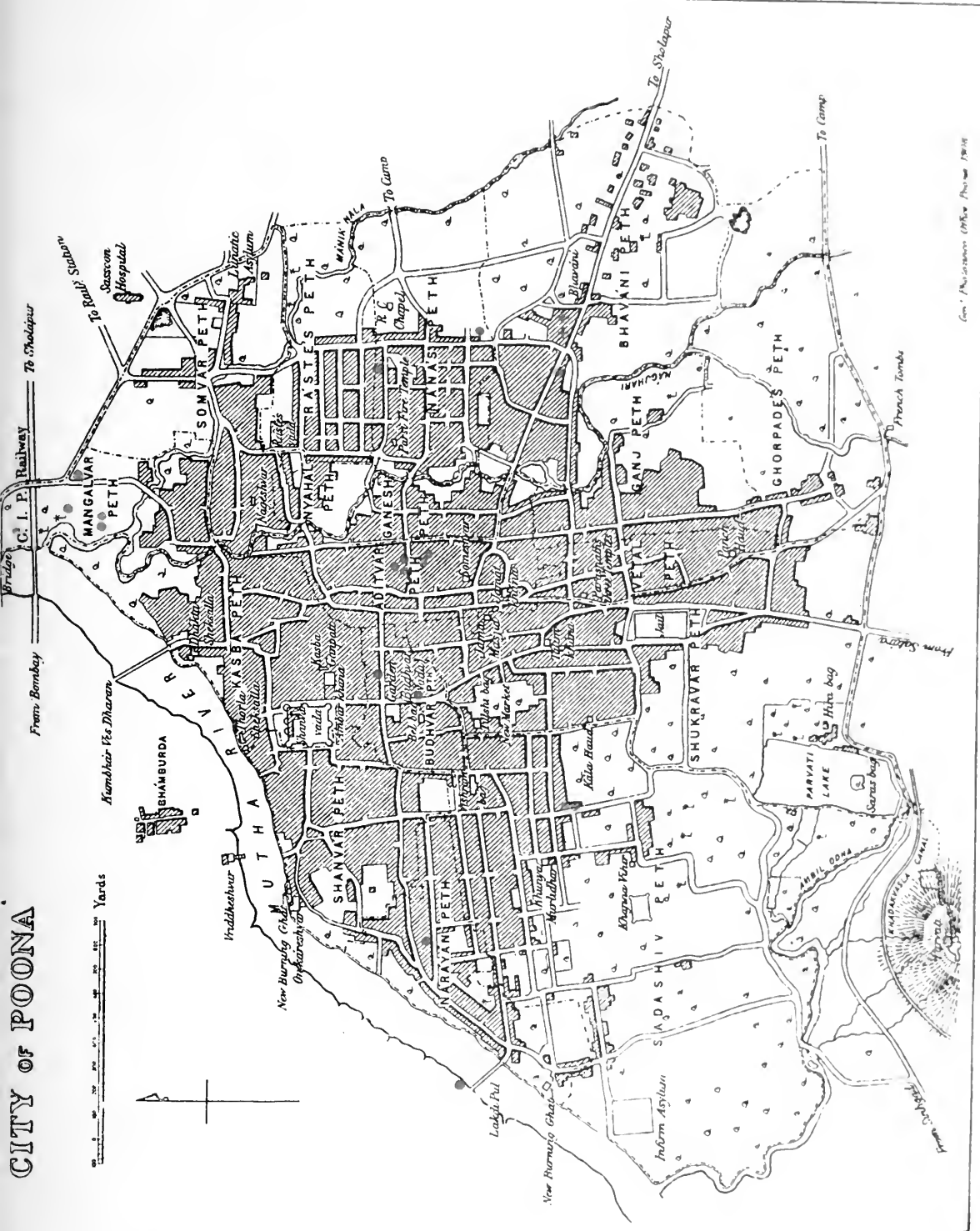
Lastly, records were kept throughout the year in the laboratory by means of a self-registering thermometer and hygrometer of variations in the temperature and humidity of the climate of Poona.

B. *The plague epidemic and epizootic of 1908—09.*

The arrangements for conducting our inquiry had scarcely been completed when already certain cases of plague came to our notice. Although, as has been remarked, on account of the extensive area covered by Poona (including in this term its suburbs) we had determined to limit our observations as far as possible to the native city, it seemed to us to be necessary to keep as close a watch as possible on the suburban districts in order to detect the first case of plague and the

¹ Petrol, especially the "Shell Brand," does equally well.

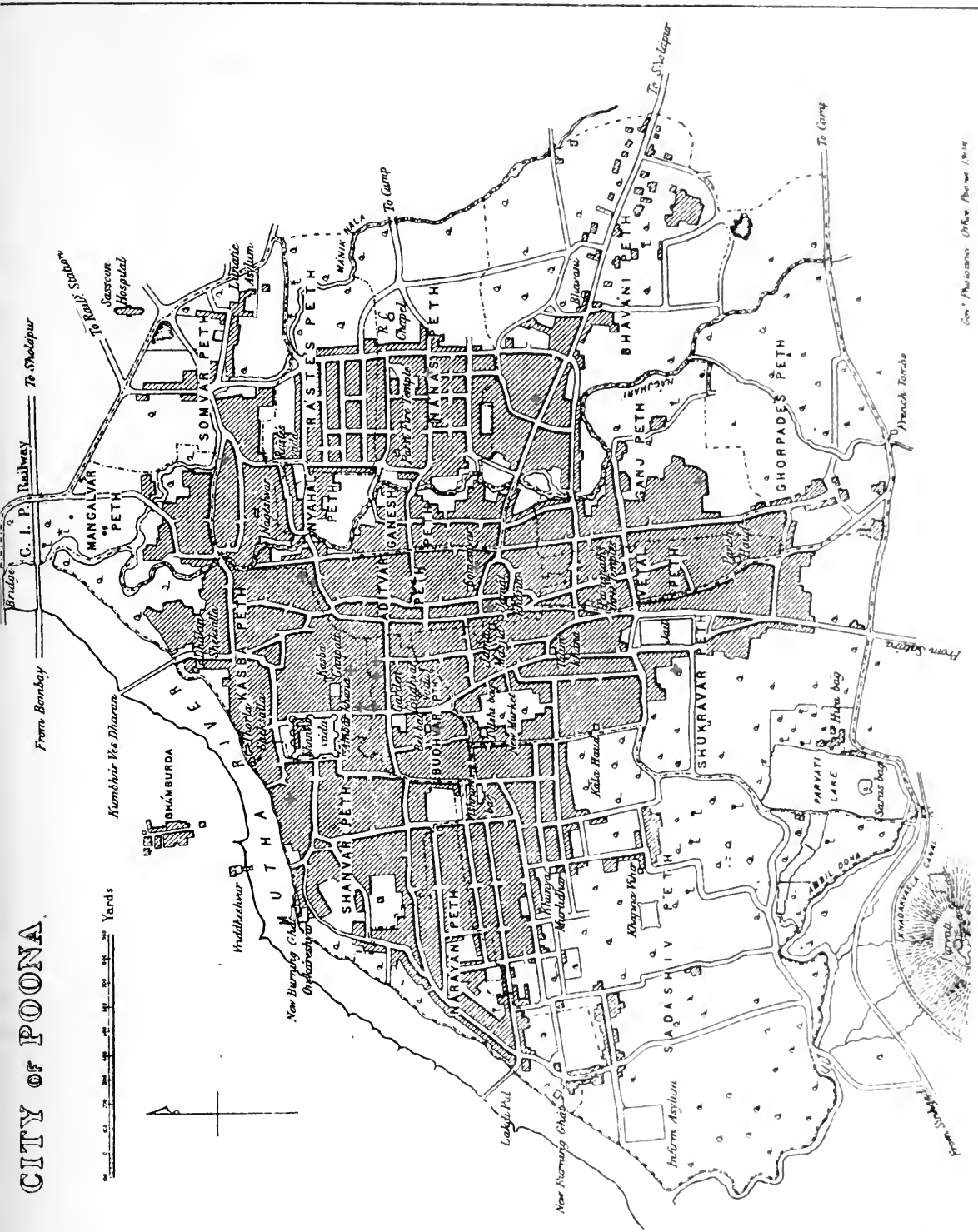
MAP III



June, July, August, 1908

● Plague cases imported

MAP IV



Plague cases reported during week ending Sept. 6. 1908.

progress of the epidemic there, at least until the disease had established itself in the city. We knew that frequent and intimate communication was continually maintained between the inhabitants of the city and the suburbs; infection, therefore, might at any time be imported from the suburbs into the city which, as our early observations showed us, was quite free from the disease, the last indigenous case having occurred in the end of January, 1908. It will be convenient to consider first the plague cases which occurred in the Suburban Municipal area. We saw that it was in this area that the railway station was situated and it was here that the first cases of plague came under our observation.

(1) *Plague in the Suburban Municipal area.* Before the beginning of June four plague cases had been imported into this area from Bombay.

May 18. 08 The history of importation was definite, for two of the cases, taken from the train, were removed to the General Plague Hospital suffering from the disease. Two other cases had quite recently come from infected areas in Bombay.

The first probably indigenous case that came to our notice occurred in the sweeper's chawl, railway servants' quarters, Poona station. A woman, about 25 years of age, the wife of a sweeper, was taken ill on the 7th June. She had a right inguinal bubo and fever and was undoubtedly suffering from plague when seen by us on the 10th June. A dead rat was said to have been found in an adjoining room in which a cow was kept, together with grass and rubbish; the rat had unfortunately been thrown away. The woman died on 12th June.

June 7 A guinea-pig, put down for some hours in the room in which the rat was said to have been found, harboured one rat flea. The guinea-pig (the flea having been replaced upon it) was removed to the laboratory where it remained healthy. The only information obtainable at the time that threw any light on the source of infection in this case was that a wedding ceremony had taken place in the house a fortnight previously, and several friends from Bombay had attended it. Later on, two more plague cases occurred almost simultaneously in the same family, a

July 4 woman of 21, and a boy of 10 years of age; they were both admitted to the plague hospital on 4th July, 1908. Sometime afterwards, on inquiring at the railway station, it transpired that, before the above cases occurred, dead rats had been found in the refreshment room of the station, and also in the signallers' quarters.

On July 6th, a man was admitted into the General Plague Hospital suffering from plague: he came from the servants' quarters of the Raj Mahal Hotel which is about 100 yards distant from the railway station:

he had returned from Bombay on 17th June, 1908, and took ill on 4th July, 1908; he had a left femoral bubo and marked aphasia. A guinea-pig was put down as a flea trap on 6th July, 1908, and 12 *L. cheopis* were caught on it; the guinea-pig remained uninfected.

Further evidence of infection in this neighbourhood was obtained whilst investigating City Plague Case No. 3. In this case the history pointed to infection having been derived from a dharmsala (a native hotel or resting house) which is adjacent to the Raj Mahal Hotel, and which is also about 100 yards distant from the railway station. The mother of the patient, a boy, who always accompanied her, used to work through the day at the dharmsala while they both slept at 719 Nana's

July 24 Peth in the city. The boy was taken ill on the 24th July and was admitted to hospital on the 28th. No evidence pointing to local infection could be obtained at 719 Nana's Peth.

City Plague Case No. 11 also possibly derived her infection at this dharmsala. She was a woman of sixty years of age, a resident of Malavadi. Passing through Poona on her way from Alandi she put up at the dharmsala adjoining the station. She was removed from the dharmsala to 235 Budhwar Peth where she died on 14th August. It is a point of some doubt whether this patient acquired her infection at the dharmsala or at Alandi; the latter place we knew to be infected for another case imported into the city acquired infection there. It may be mentioned that Alandi is a place about fourteen miles north of Poona, greatly frequented at all times by pilgrims from every part of the surrounding district.

A visit to the dharmsala showed that it was frequented by a poor class of people generally passing through Poona. Only a vague history of dead rats was obtained; the manager, unwilling to bring disrepute on his establishment or fearing to cause a panic amongst his clients, was very reticent nor would he allow us to apply the guinea-pig test for infection.

On August 29th, some dead and partially mummified squirrels were found in the roof of the office of the Inspector-General of Aug. 29 Police which was about $\frac{1}{4}$ mile to the south-west of the station. The place was evacuated. A guinea-pig was put down but no fleas were found on it and it remained healthy.

On September 4th three dead rats were found in the goods sheds of the G.I.P. Railway about $\frac{1}{4}$ mile to the west of the station. September 4 These were proved to be plague infected and subsequently others were found in this neighbourhood.

On September 21st two dead rats were sent from the Lunatic Asylum which is just within the city boundary. The September 21 asylum is about $\frac{1}{4}$ of a mile south-west of the Inspector-General's office mentioned above. These rats were also proved to be plague infected.

Such then is briefly the early history of the epidemic and epizootic in the Suburban Municipal area. It apparently started in the neighbourhood of the station, the rats acquiring the disease from infection probably imported by the railway. It spread thence in a westerly and south-westerly direction towards the city. That the epidemic did not assume greater proportions with the advent of favourable conditions may be explained by the fact that in this neighbourhood all the houses are of the better class, viz. government offices and private bungalows with extensive compounds surrounding them and widely separating them from each other. Meanwhile plague cases were reported from the Kirkee Cantonment; the history of this epidemic and epizootic will next be considered.

(2) *Plague in Kirkee Cantonment.* Two cases of plague were May 30 admitted to the Cantonment Hospital on the 30th May, June 2 while a third case was admitted on the 2nd June. All three cases lived in separate houses and apparently were in no way connected with one another, nor was it possible to trace the source from which they derived infection; certain it is that for eighteen months prior to these cases none were known to the authorities.

No further cases occurred till the 28th June, when two cases living June 28 in the same house occurred quickly following one another, the one on the 28th June and the other on the 1st July. Thereafter the epidemic became widespread throughout the Kirkee Cantonment bazaar, 65 deaths from the disease being registered in July while 128 were recorded in August. In the three succeeding months the epidemic rapidly declined with 68 deaths in September, 21 in October, and only two in November. In all there were 288 deaths in a population of 5640. Bearing in mind that a very large part of this population consisted in British and Indian troops among whom very few cases occurred, the epidemic cannot but be regarded as a very severe one. We shall later offer an explanation for the severity of the epidemic, meanwhile contenting ourselves by referring the reader to Table VII which compares the Kirkee epidemic with the epidemics in Poona City, Poona Cantonment and the Suburban Municipal area.

Observing that the epidemic raged in Kirkee while it had hardly begun in the city, we determined to collect and examine a certain number of rats from this locality in the same manner as we were examining those collected in the city. These operations were started on the 17th July, and on the 21st July, four days after trapping was begun, a plague infected rat was found among those caught on that day. On the 27th another plague infected rat was captured in No. 2 Battery Lines and thereafter several others.

TABLE VII.

Table showing the number of plague deaths per mille of population in Poona City, Cantonment, Kirkee, and Suburban limits in monthly periods during the year 1908—09.

Month	Poona City Population 1901—111,381		Cantonment Population 1901—32,777		Kirkee Population 1901—5640		Suburban limits Population 1901—9162	
	Deaths	Per mille death rate	Deaths	Per mille death rate	Deaths	Per mille death rate	Deaths	Per mille death rate
June '08	—	—	—	—	4	·71	—	—
July	4	·04	—	—	65	11·11	1	·11
August	9	·08	10	·305	128	22·695	5	·545
September	123	1·10	71	2·17	68	12·06	14	1·53
October	472	4·24	142	4·33	21	3·72	31	3·84
November	370	3·32	116	3·54	2	·355	16	1·75
December	191	1·71	29	·885	—	—	2	·22
January '09	101	·91	4	·12	—	—	1	·11
February	21	·19	1	·03	—	—	—	—
March	3	·03	2	·06	—	—	—	—
April	1	·01	—	—	—	—	—	—
May	2	·02	—	—	—	—	—	—
June	—	—	—	—	—	—	—	—
Total	1297	11·645	375	11·44	288	51·06	70	7·64

An average of about 20 rats were brought in daily—397 in all were examined, of which 364 were alive and 33 dead—of these 10 live rats and 22 dead ones were found to be plague infected.

The observations were discontinued at the end of August, when it was found very difficult to catch any more rats, and when, moreover, our attention had become fully occupied by the city epidemic which had just commenced.

Tables VIII and IX summarise the observations made in Kirkee in respect to (1) the number of fleas found on the rats, and (2) the number of rats caught per 100 traps set, and compare these figures

with similar ones obtained from Poona City during the same periods. It will be seen from these tables (1) that the numbers of fleas found per rat in each place do not materially differ from one another, although the fleas on the rats were slightly more numerous in Kirkee than in Poona; (2) that while, on the one hand, the number of rats caught per 100 traps set in Poona city in the latter half of July was not only higher than the number caught in Kirkee but was increasing each fortnight later, on the other hand, the number of rats caught in Kirkee rapidly dropped each fortnight later, from 32·2 in the latter half of July, when the epizootic was already well started, to approximately only nine, when the observations were discontinued, that is when the epidemic was rapidly declining. The conclusion to be drawn from these observations are better left for consideration later. We may pass on now to briefly outline the course of the epidemic in the Poona Cantonment.

TABLE VIII.

Table comparing the number of fleas per rat in Kirkee and Poona City for corresponding periods.

Period	Poona City			Kirkee		
	Flea counts made on live <i>rattus</i>	Total no. of fleas obtained	Average no. of fleas per rat	Flea counts made on live <i>rattus</i>	Total no. of fleas obtained	Average no. of fleas per rat
July 17 to 31 '08	2304	13100	5·7	215	1337	6·1
Aug. 1 to 31 '08	4700	42871	9·1	129	1355	10·5

TABLE IX.

Table comparing the number of rats per 100 traps set in Poona City and Kirkee Cantonment during July, August, September in fortnightly periods.

Fortnight ending	Poona City			Kirkee Cantonment		
	No. of traps set	No. of rats caught	No. of rats per 100 traps set	No. of traps set	No. of rats caught	No. of rats per 100 traps set
July 26 '08	6614	2449	37·04	371*	123	33·2
Aug. 9 '08	6996	2858	40·9	672	108	16·1
Aug. 23 '08	6558	2733	41·6	672	61	9·1
Sept. 6 '08	6816	2726	40·0	336†	33†	9·8†

* These are figures from 19th to 26th July only.

† These are figures from 24th to 30th Aug. only.

(3) *Plague in Poona Cantonment.* A case of plague occurred in the Poona Cantonment on the 27th July. This case was said to have acquired infection in Kirkee. It was soon followed however by one of local origin reported on the 1st August. Ten cases occurred during that month at fairly regular intervals. During the first fortnight of September, 55 were reported. In the first half of November 126 cases were reported, and in the second half only 36. The epidemic then declined till the end of December. We were unable to give much attention to the study of plague in the cantonment, for the epidemic in the city commenced soon afterwards, the first indigenous cases in the city being registered on the 28th August, while the first plague infected rat (apart from a plague infected mouse found on the 18th July) was noted on the 7th Sept.

(4) *Plague in Poona City.* (a) *The epidemic.* While thus the epidemic raged in the suburbs of Poona, especially in Kirkee, not till the end of August could any evidence of indigenous plague be obtained in the city. Large numbers of people meanwhile were flocking into the city from the surrounding infected areas to live there with friends or relatives in uninfected quarters, if haply they might escape the plague. One who is familiar with the habits of Indians can easily picture the flight from the infected suburbs; whole families, carrying with them their household impedimenta and oft times their sick relations, might be seen on the roads leading to the city. In reviewing the course of the epidemic in the city, one would expect to find therefore, first a number of imported cases of the disease, occurring in widely scattered localities: then later a large number of indigenous cases, also widely scattered, arising from numerous infected centres. If, on the one hand, the disease is infectious (using the word infectious in the ordinary acceptation of the term) it would be right to expect that the indigenous cases would occur in direct association with the imported ones; on the other hand, if infection is transmitted indirectly from rat to man and from rat to rat by the agency of the rat flea then it would be reasonable to expect that, in many instances, the indigenous cases would bear no immediate relation to the imported ones; moreover many of the indigenous cases might occur in localities where the source of the infection could not be traced, especially when we bear in mind the fact that rat fleas are readily carried by man, almost unknown to himself, from one place to another, and that the fleas by choice select rats for their hosts rather than man. Centres of epizootic infection arise in this way in areas where a history of the importation of the

infection could only be obtained with the greatest difficulty. The history of the epidemic in the city will be seen to bear out this latter hypothesis.

We have detailed in another part of this report the means we adopted to inquire into the history of the first hundred cases of plague in the city. These careful inquiries enable us to state with some confidence that the first eighteen cases of plague which occurred in the city acquired their infection outside of it. A review of these cases showed that they were attacked by the disease at dates between the 1st July and the 24th August. Fourteen of the cases picked up infection in the suburbs of the city, ten in Kirkee and four in the municipal area. Four other cases acquired infection away from Poona and came by rail to the city, two from Kalyan (a place near Bombay), one from Bombay and one from Alandi, a place already mentioned in connection with the epidemic in the municipal area. Following these imported cases, the first indigenous case took ill on the 24th August; the patient, a man, had never left the city, nor had he been in contact with any plague cases. The nearest imported case occurred in a house two hundred yards distant. This imported case had acquired infection in Kirkee whither he had gone on a visit; returning therefrom on the 27th July, he developed plague the same day and died on the 29th. Two days after the first indigenous case was attacked by the plague (*i.e.* the man attacked on 24th August) his wife fell ill with the disease and died on the 29th August, the day after her husband's death. This woman seldom left her house and without doubt acquired infection there.

On September 5th thirteen cases were reported as having occurred during the previous five days, in various parts of the city, viz. at Rawiwar 50, Kasba 40, Kasba 195, Kasba 5, Rawiwar 591, Shukurawar 293, Budhwar 429, Bhawani 951, Rawiwar 1082, Nana 287, Budhwar 426, Somwar 368; with the exception of the first two houses all are rather widely separated from each other.

It is worthy of note here that Kasba Nos. 40 and 5 and the two houses in Budhwar are not very far removed from the house in which a partially decomposed mouse (which was proved to be plague infected) was found on the 18th July. We may also mention here, that on September 7th we obtained further evidence of the presence of an epizootic in this neighbourhood by finding a dead plague infected rat at No. 5 Kasba, while on the 8th a live rat suffering from plague was trapped in house No. 1419 Kasba, this house being about five hundred yards distant from the above mentioned No. 5.

On September the 6th four more cases were reported to us as having occurred during the last few days at Gang 363, Shanwar 27, Budhwar 169, and another case was brought to the General Plague Hospital from an address in Shanwar Peth, which could not be traced.

We may at this stage direct attention to two maps which show the distribution of the early cases of plague. Map III shows the position of the houses in which the first twenty cases occurred; all of these, except two which were infected in the end of August, were imported cases of the disease occurring during the months of July and August. Map IV shows the distribution of the first indigenous cases of plague (save the two above mentioned) which occurred in the first week of September. From these maps it will be gathered that the indigenous cases bore no relation, at least as regards distribution, to the imported ones, and from Map IV the inference can be made that already, early in the epidemic, foci of indigenous plague were widely distributed through the city.

In order that we might be assured that no deaths from plague had been overlooked, the daily death returns of the Health Office were obtained, and a note made of all deaths which occurred during July and August between the ages of three and sixty years, excluding only those returned as deaths from plague. The places where these deaths had occurred were then allocated on a large scale map of the city with a view to selecting those for further inquiry which appeared to be grouped particularly closely together. This inquiry was made by personal visits to the friends of the deceased with the object of eliciting any facts which might point to the deaths as having been due to plague but incorrectly registered under another cause. Two areas only fell under suspicion, viz. Budhwar and Nana's Peths.

Accordingly 23 deaths in Budhwar and 21 deaths in Nana's Peth were investigated. Amongst other details of the case, the history and chief symptoms were noted, viz. the presence of delirium, fever, glandular swellings, etc; also the duration of the illness; presence, at the time, of a rat mortality, or other cases of plague in the neighbourhood, and whether the patient had been treated by a medical man, in which case further inquiries were also made from him. The homes of seven persons who had died in Budhwar Peth could not be traced, of the other deaths fifteen were definitely due to a chronic illness of from a month to a year's duration. The remaining death occurred at 443 Budhwar. This person was taken ill on 26th August with, the friends said, fever and a rigor, followed by diarrhoea and vomiting lasting two

days, the death had been registered as due to diarrhoea, but on inquiring from the medical practitioner who treated the case he describes it now as definitely due to plague. This case occurred at a house not far from the one in which the first plague infected mouse had been found on July 18.

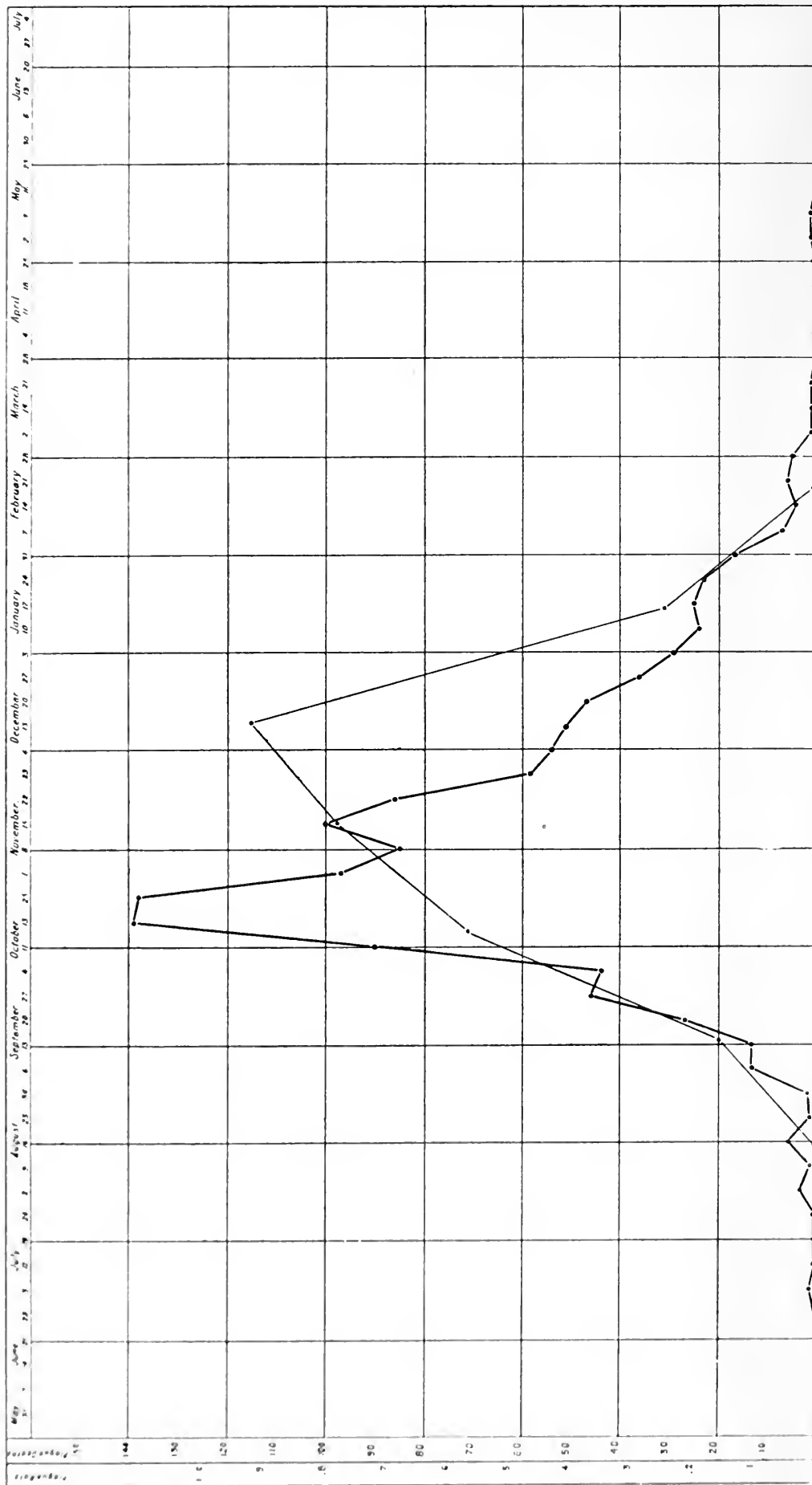
In a similar way twenty-one deaths in Nana's Peth were investigated. The homes of three of the deceased could not be traced. The cause of death in the remaining cases except three was due to chronic illnesses of some kind. Of the three deaths due to acute illnesses, one appeared to have died from cholera, another suffered from an illness of fifteen days' duration following parturition without any history pointing to plague. The remaining case a Mahomedan boy, aged 12, took ill on 19th August at No. 288 Nana's Peth. The illness started with a rigor and fever; no glandular swelling was observed. The boy died on August 25th, 1908. The neighbours, however, described this as definitely a plague case, and remembered noting other cases later in the same locality. The house, since the death occurred in it, has remained unoccupied.

The result of this inquiry showed that, on the one hand, we probably did not miss many plague cases during the early part of the epidemic, on the other hand, we might with equal certainty say that one or two cases of plague did occur, which, for various reasons, had been kept hidden from the authorities.

From the 6th September onwards the number of deaths from plague rapidly increased, so that by the end of September there had been 123 deaths from this cause followed by 472 in October. The preventive measures which we have already detailed in another part of this report were meanwhile being actively pushed, so that in November there was already a distinct fall in the mortality from plague in the city, three hundred and seventy deaths being recorded in this month, this, be it noted, in spite of the fact, as will be evident by consulting Table X, that the epizootic among the rats was still on the increase. In December there was a still greater fall in the number of deaths from plague, which in this month registered 191. In January, February, and March the figures were respectively 101, 21 and 3. The epidemic came to an end with four imported cases of the disease, one in March, one in April and two in May.

(b) *The epizootic.* We have seen that the first evidence of plague among the rodents of the city was obtained in the finding of a plague infected mouse on the 18th July. It seems probable that, at least in

CHART V



POONA

Plague deaths in Poona City per week: 1907-1908

the locality where this mouse was found, we succeeded in obtaining evidence of the very commencement of the epizootic, for some time elapsed before further testimony was forthcoming of the presence of the disease in the place either in the return of plague cases (the earliest possible one being the doubtful plague case registered as cholera on the 26th August at 443 Budhwar Peth mentioned above) or in the discovery of plague infected rats, the first one being found on the 7th September; the epizootic however by this latter date appeared to be fairly widely distributed in the neighbourhood, for on the following day (8th Sept.) a plague infected rat was trapped in a house more than a quarter of a mile distant. Thereafter the epizootic rapidly increased.

TABLE X.

*Table showing percentage of live rats (*Mus rattus*) plague infected (acute) in monthly periods, during the year 1908—09.*

Period	Number of live <i>Mus rattus</i> examined	Number of live <i>Mus rattus</i> (acute) plague infected	Percentage of live <i>Mus rattus</i> plague infected	Resolving plague	Percentage	Plague deaths in Poona City
Sept. '08	6115	10	0·16	18	·2	123
Oct.	4913	29	0·59	27	·5	472
Nov.	3814	30	0·78	29	·7	370
Dec.	2924	27	0·92	77	2·6	191
Jan. '09	2376	6	0·25	33	1·0	101
Feb.	2284	1	0·04	22	·9	21
March	2906	0	—	18	·6	3

In view of the fact that dead rats were received at the laboratory at very infrequent intervals, we determined to consider only those rats which were trapped alive and found to be infected with plague in measuring the onset, progress, and termination of the epizootic. In adopting this course we no doubt considerably curtailed the actual period during which the epizootic prevailed, for the reasons that (1) the number of rats examined daily, on an average two hundred, a representative sample no doubt as far as distribution was concerned, was only a very small fraction of the whole of the rats in the city, and (2) the chance of capturing a plague sick rat by tempting it into a trap with bait was probably small; yet this method yielded results which appeared to be near the truth. It is apparent from what has been said above, that the capture of the first plague infected rat occurred only about a fortnight

(15 days) after the first indigenous case fell ill with the disease. Considering then only rats caught alive in the city suffering at the time from acute plague we found ten in September, twenty-nine in October, thirty in November, twenty-seven in December, six in January, and one in February; these numbers are detailed in Table X where also the percentage of plague infected rats on the total number examined has also been calculated, and these figures compared with the number of deaths from plague that occurred among the inhabitants of Poona City each month. We think that we have measured the extreme limits of the epizootic rightly when we state that it lasted from the end of August to the end of March and that it reached its acme in the month of December.

It is convenient here to mention certain other features connected with plague among the rats of Poona. In previous reports we detailed at some length the naked eye pathological appearances found in acute plague infected rats, which had died of the disease. In Poona and Belgaum we frequently encountered the disease in living rats; it was natural therefore that we should find all stages of the disease. Some rats, for example, had just acquired infection; the pathological changes in these cases were of course somewhat obscure, sufficient time not having elapsed from the commencement of the disease to allow the lesions to be easily visible to the naked eye; a few of these rats therefore might have escaped detection during dissection had it not been for a microscopical examination of a spleen smear, in which it was possible, even at this early stage, to detect plague bacilli. Again, other rats were met with which had apparently passed the critical stage of the disease, and which, had they not been captured and killed, might well have recovered from the disease. Among this latter group of plague infected rats all stages of recovery from the disease were met with. There were rats, for example, with lesions closely resembling those found in acute plague, but on closer inspection these lesions were observed to be more isolated, and more localised in the tissues. Thus the presence of minute necrotic spots in the liver is a very characteristic pathological finding in rats which have died from acute plague; on close inspection these necrotic spots are found to have an ill-defined margin, the necrotic material fading away imperceptibly into the liver tissue, but in rats which are recovering from the disease these necrotic areas have a more defined outline, are often larger, and less numerous. Then, again, there were rats also belonging to this group, apparently also recovering from

the disease, which presented the lesions of resolving plague described elsewhere (p. 335). Finally, a number of rats were observed, which showed various scars and adhesions between the abdominal viscera, the parietes and mesentery; these rats we believe had suffered from plague, but had recovered. The importance of the existence of these cases of resolving and recovered plague among the rats will be appreciated when we come to discuss the question of immunity among rats as a factor in causing the decline of an epidemic.

TABLE XI.

Name of month	Total live <i>rattus</i> examined	Acute plague		Resolving plague		Adhesions	
		No.	Percentage	No.	Percentage	No.	Percentage
June 1908	3977	0	—	3	—	Figures not available before November.	—
July	5351	0	—	4	—		—
Aug.	5946	0	—	4	—		—
Sept.	6115	10	·16	18	·2		—
Oct.	4913	29	·59	27	·5		—
Nov.	3814	30	·78	29	·7		—
Dec.	2923	27	·92	77	2·6	26	·88
Jan. 1909	2376	6	·25	33	1·0	43	1·8
Feb.	2284	1	·04	22	·9	57	2·4
Mar.	2906	0	—	18	·6	48	1·6
April	2644	0	—	5	—	28	1
May	2479	0	—	5	—	19	·7
June	2830	0	—	4	—	22	·7

C. *Data bearing on the factors which determine the seasonal prevalence of plague collected in Poona 1908—1909.*

(1) *The climatic conditions in Poona 1908—1909.*

Under this head little requires to be added to what has already been said. From a study of Charts I, II and III it will be appreciated that the climatic conditions of the year approximated very closely to the normal. In Charts I and II the mean daily temperature, averaged for weekly periods, for the year 1908—09 may be compared with the mean daily temperature, averaged for half-monthly periods, and calculated for the ten years 1897—1906. In Chart II the relative humidity, expressed in percentage of saturation, calculated on six hourly readings daily and averaged for each week, has been compared with similar figures

expressing the mean result for the ten year period 1897—1906. Chart III shows the maximum, minimum, and mean temperatures, averaged for weekly periods, for the year 1908—09.

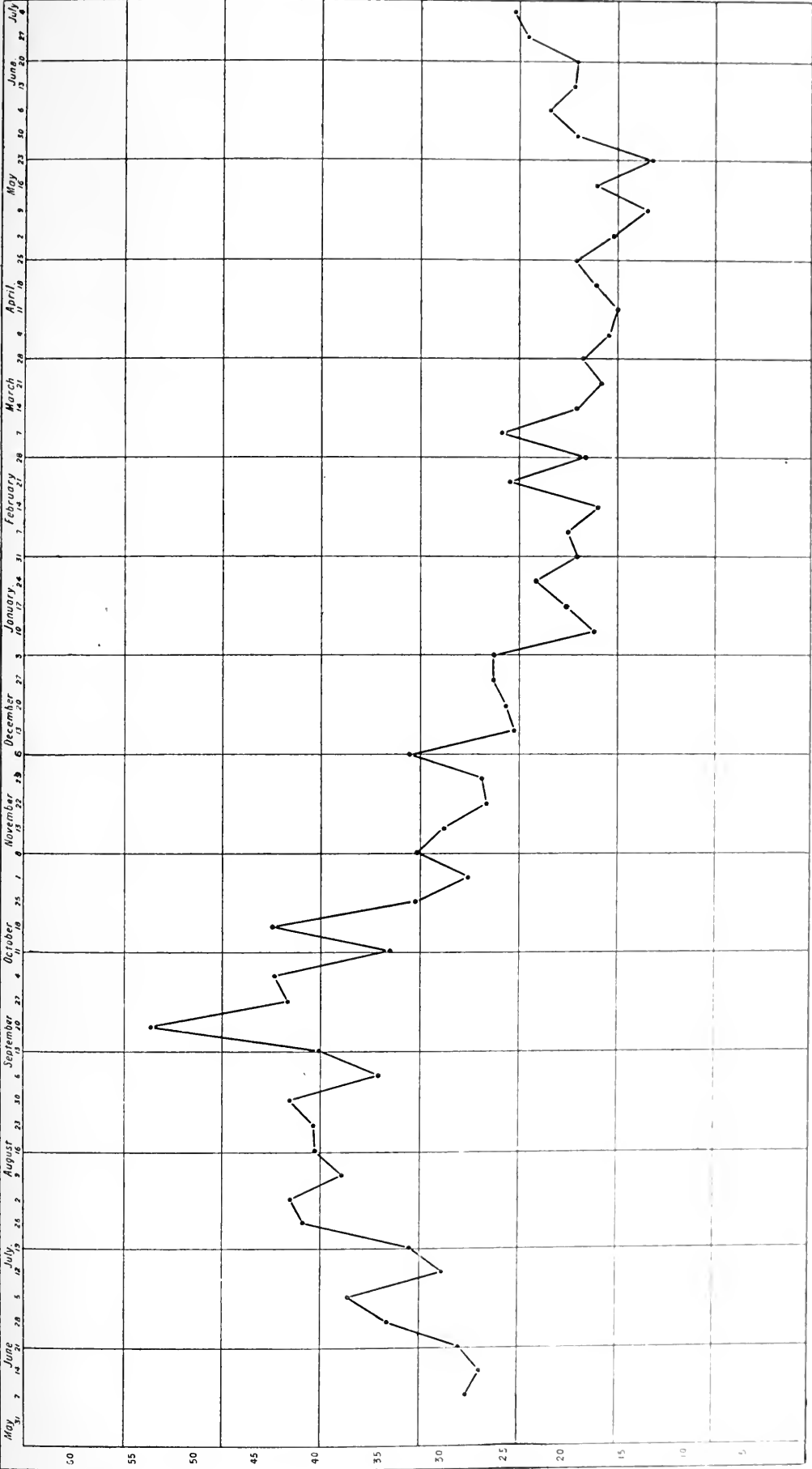
(2) *Variations in the virulence of the bacillus.*

Practically no data have been collected under this head. In the absence of any satisfactory standard for measuring the virulence of the plague bacillus little can be said on this subject; it will suffice to mention, that strains of the plague bacillus were isolated in Poona, during the course of the epidemic, which showed a high degree of virulence.

(3) *Variations in the total number of rats.*

The means by which we hoped to collect data bearing on the infestation of the houses with rats in Poona City have already been discussed. We hoped by setting in rotation a number of rat traps daily in the houses of the city to be able to measure their rat infestation by calculating the number of rats caught for every one hundred traps set. The following table shows the total number of rodents and "musk" rats caught during these operations from May 1908 to June 1909. It will be observed that the number of rats caught amounts to approximately one half of the population of the city. It will also be noted that the species *M. rattus* predominates largely over the other species; we have considered this species only, to the exclusion of the others, in conducting our inquiry.

Bearing in mind the limitations which must be placed on the use of our method for estimating rat infestation, a method open to many fallacies despite our best endeavours to avoid the most obvious sources of error, we think our figures are trustworthy enough to show that there was, during the year, a marked variation in the rat infestation of the houses in the city. The figures we have compiled can be seen in Table XII; it will be noted that the number of rats caught per 100 traps set during the first four months of the operations steadily increased from 30·7 in June to 42·8 in September; meanwhile more than twenty thousand rats had been caught, this considerable destruction of rats having little effect in reducing the rat infestation of the houses when measured by our method. It will be seen from Table XII that breeding among the rats was going on actively during this period,



POONA

Number of rats per 100 traps set in Poona City

TABLE XII.

Table showing the number of rats caught in Poona City per 100 traps set.

Period	Number of traps set	Number of rats caught	Number of rats caught per 100 traps set	Remarks
June 1st to June 28th	11363	3494	30·7	
June 29th to July 26th	13249	4704	35·5	
July 27th to Aug. 30th	16888	6890	40·8	
Aug. 31st to Sept. 27th	12697	5436	42·8	Epizootic begun.
Sept. 28th to Oct. 25th	11620	4508	38·8	
Oct. 26th to Nov. 29th	14862	4283	28·8	
Nov. 30th to Dec. 27th	9036	2471	27·3	Acme of epizootic.
Dec. 28th to Jan. 31st	11178	2370	21·2	
Feb. 1st to Feb. 28th	10479	2116	20·2	
Mar. 1st to Mar. 28th	12907	2562	19·8	Epizootic ended.
Mar. 29th to April 25th	14657	2533	17·2	
April 26th to May 30th	18606	2966	15·9	
May 31st to June 27th	13295	2775	20·8	

Table showing percentage of young rats on total rats weighed.

Period	Number of rats weighed	Number of young rats	Percentage of young rats under 70 gms.	Remarks
June 1st to June 28th	3479	1916	55·1	
June 29th to July 26th	4640	2481	53·4	
July 27th to Aug. 30th	6740	3398	50·4	
Aug. 31st to Sept. 27th	5736	2895	50·5	Epizootic begun.
Sept. 28th to Oct. 25th	4782	2557	53·4	
Oct. 26th to Nov. 29th	4525	2211	48·8	
Nov. 30th to Dec. 27th	2697	1054	39·0	Acme of epizootic.
Dec. 28th to Jan. 31st	2797	1082	38·6	
Feb. 1st to Feb. 28th	2301	1090	47·3	
Mar. 1st to Mar. 28th	2613	1485	56·8	Epizootic ended.
Mar. 29th to April 25th	2422	1451	59·9	
April 26th to May 30th	2890	1670	57·7	
May 31st to June 27th	2728	1443	52·8	

Table showing percentage of pregnant females.

Period	Adult female <i>rattus</i>	Female <i>rattus</i> pregnant	Percentage of adult females pregnant	Remarks
June 1st to June 28th	937	366	39·1	
June 29th to July 26th	1282	551	42·9	
July 27th to Aug. 30th	2149	870	40·4	
Aug. 31st to Sept. 27th	1702	653	38·3	Epizootic begun.
Sept. 28th to Oct. 25th	1302	384	29·4	
Oct. 26th to Nov. 29th	1271	304	23·9	
Nov. 30th to Dec. 27th	833	184	22·1	Acme of epizootic.
Dec. 28th to Jan. 31st	929	307	33·0	
Feb. 1st to Feb. 28th	668	236	35·3	
Mar. 1st to Mar. 28th	632	248	39·2	Epizootic ended.
Mar. 29th to April 25th	562	214	38·1	
April 26th to May 30th	696	283	40·6	
May 31st to June 27th	730	297	40·6	

CITY of POONA



TABLE XII (continued).

Table showing total rodents caught.

Mus Rattus	49,678
Mice	686
Musk Rats	37
Gunomys varius or Nesokia bengalensis	1
Total				50,402

In addition to the animals mentioned above sixteen mongooses were caught in the rat traps; the mongooses had apparently entered the traps in order to kill the rats caught in them, and were chiefly taken during the rainy season. We may here also mention two facts of subsidiary importance, viz. (1) no bandicoots (*N. bandicota*) were seen or captured in Poona, but intelligent citizens declare that, before plague visited Poona, rats answering this description were commonly seen there; (2) no *M. decumanus* were caught in Poona, contrasting markedly in this respect with Bombay, and this in spite of the fact of the proximity of Bombay to Poona, and that there exists in this city a system of underground drains or closed gutters suited to the habits of *decumanus*; we would infer therefore that this species of rat is not very commonly transferred from one place to another in merchandise. The local distribution of the variety *alexandrinus* is shown in Map V.

About 40% of all females captured being pregnant. The number of young rats among the total number of rats caught at this time was also considerable; from Table XII it will be seen that more than 50% of all the rats captured weighed less than 70 grams.

From the end of September to the end of January there was a very rapid decrease in the number of rats caught per 100 traps set. It is possible that a number of factors contributed to bring about this decrease. First among these we must mention the plague epizootic which came to our notice, be it remembered, in the discovery of plague infected rats in the beginning of September, and which thereafter rapidly developed, reaching its acme in December. During this period, September to the end of December, there was a very marked drop in the number of rats caught per 100 traps set, from 42·8 to 27·3, a difference of 15·5 rats per 100 traps set. With the decline in the epizootic a considerable, though diminished, fall was still maintained, especially during the first month of the decline, the number of rats caught decreasing then from 27·3 to 21·2 per 100 traps set; later, a more gradual decline took place to the close of the epizootic in March, when 19·8 rats were caught. There can be little doubt, therefore, that the epizootic had a considerable effect in reducing the rat infestation of the houses. We saw that in Kirkee, where the epizootic was much more severe than it was in the city, an even greater reduction in rat infestation took place from this cause

during the short period of one and a half months, viz. from 33 rats per 100 traps set to approximately nine.

We must not however overlook the fact that during the epizootic period, at least from the end of August to the end of December, there was a very considerable curtailment in breeding among the rats, and this must also have had some effect in bringing about the fall in the number of rats caught recorded above. The percentage of females found to be pregnant fell during this period from 40·4% in the end of August to 22% in the end of December. Whether this decrease in the number of pregnant females was directly due to the epizootic or not, we cannot at present say; future observations, during a year when plague is absent from the city, may shed some light on the matter. After the month of December, however, which, curiously enough, corresponded exactly with the acme of the epizootic, the breeding again increased. Following the slack breeding period in September to November, there was a reduction in the percentage of young rats among the total rats captured, the figures falling, as will be seen in the table referring to this matter, from 53·4% in October to 38·6% in January; thereafter, the percentage of young rats among the total rats again increased.

By the end of January, therefore, conditions again seemed to be favourable for an increase in the rat infestation of the houses, the epidemic was on the decline, more females were pregnant, and more young ones were being added to the rat population, but our figures do not show any increase in the number of rats caught per 100 traps set. There is rather a decline, for, after remaining at a fairly constant, though declining level, during January, February and March, when 21·2, 20·2 and 19·8 rats respectively were caught for every 100 traps set, a still further and rather greater drop in the figures occurred during April and May when, so far as we were aware, no epizootic existed, and when only 17·2 and 15·9 rats were caught.

To account for this persistent fall in the number of rats caught in spite of circumstances favouring their increase, such as active breeding and the absence of epizootic disease, we must presume that either (1) the reduction in the number of rats brought about through the combination of factors mentioned above had been so great, that now our trapping operations (being still maintained nearly as actively as at the commencement, when we saw they had little effect on the rat infestation), were causing the destruction of a number of rats as great, if not greater than, the number which was being added to the rat forces

by breeding, or (2) some other factor, which we have not yet considered, was playing a part, directly or indirectly, in reducing the number of rats captured. In seeking for this other factor we cannot help being struck by the fact that the rat infestation of the houses was maintained at a high level during the rainy season, and gradually declined from the termination of the rains to the close of the hot weather, when again, with the advent of the rains, an increase in the number of rats captured was immediately registered. On the one hand, it is reasonable to suppose that the inclement weather conditions during the rains might tend either to drive rats which lived outside of houses to find shelter in them during this season or that it tended to cause rats, which habitually lived in the houses but sought for food outside, to look for it now within their sheltered homes, and so cause them more frequently to be caught in our traps at this season, the traps, of course, being always set within the houses. On the other hand, it seems equally reasonable to suppose, in view of the fact that the rainy weather almost abruptly terminates in the end of September, that the tendency for the rats to leave the houses would occur almost as abruptly; nevertheless, in place of an abrupt egress, our figures show that a long, slowly drawn out migration from the houses to the surrounding compounds must be supposed to take place. If there is such a seasonal migration of the rats into the houses during the rains and out again during the dry weather it must be considered as a factor favouring the prevalence of plague during the rainy season in Poona, for then the rats, which are known to play so important a part in conveying the disease to man, would on this hypothesis be more intimately associated with him than at any other time of the year.

(4) *Variations in the proportion of immune to susceptible rats.*

An experiment was carried out in Poona during the course of these observations to test the immunity of rats during the progress of the epizootic. From 70 to 100 rats every week throughout the year were inoculated with as constant a dose as possible of a virulent strain of plague bacillus to ascertain the proportion that died of plague, and in this way to get a measure of the immunity of the rats to plague infection. For the purpose we had in view the experiment entirely miscarried, chiefly for the following reasons. (1) During the damp rainy season it was difficult to keep dry the cages in which the rats were kept,

so that many rats died from causes other than plague. (2) During the cold weather, especially in November and December when the minimum temperature during the night frequently fell to below 50° F., many rats were found in the early morning to be in a moribund condition; some of these died and were found to be suffering from plague, others however revived when the temperature rose later in the day. The lowering of the temperature during the night was apparently so important a factor in increasing the liability of the rats to death after inoculation with plague, in the artificial surroundings in which the rats had to be kept, as to obscure any increased immunity they might have acquired in having passed through an epizootic of plague. It was found impossible to avoid this source of error in conducting this experiment, for the rats had to be kept in cages in an open corrugated iron shed which occupied a somewhat exposed position; moreover the full significance of this source of error was not at the time recognised. The experiment however suggests that the cold weather may be a factor in increasing the death rate among plague infected rats.

We have therefore to fall back on indirect evidence as to whether during the progress of an epizootic of plague, especially towards its close, a large number of rats acquire immunity to the disease. The evidence of this sort which we were able to collect in Poona may be seen in Table XI, where we give the number of rats found each month during the epizootic suffering from "resolving plague," *i.e.* an attack of plague from which the rats were evidently recovering; in this table, it will be seen that as many as 2·6, 1·0 and ·9% of the rats caught alive in the city during the months of December, January and February respectively presented this form of the disease.

(5) *Variations in the number of fleas found on rats.*

Loemopsylla cheopis was practically the only flea found on the rats of Poona. Amongst 240,433 fleas caught throughout the year, only two cat fleas and four human fleas were seen. The average number of fleas found on *Mus rattus* was noted from week to week (Table XIII). When observations were commenced in May 1908, and until the week ending June 21st, their number appeared to be still on the decline. In that week they reached the minimum for the year of 1·3 fleas per rat. A rapid and very constant rise then took place, until the week ending August 30th, when the maximum for the year, *i.e.* 11·2 per rat, was

TABLE XIII.

Table showing the average number of fleas per rat (*M. rattus*)
in weekly periods.

Week ending	Number of <i>rattus</i> on which fleas were counted	Number of fleas counted	Average number of fleas per rat
June 7, 1908	640	1370	2.1
14	663	1826	2.8
21	886	1194	1.3
28	1115	2789	2.5
July 5	1181	4317	3.7
12	951	3761	4.0
19	1063	5622	5.3
26	1152	5725	5.0
Aug. 2	1030	7117	6.9
9	1026	7902	7.7
16	1010	8836	8.7
23	1112	10274	9.2
30	1168	13052	11.2
Sept. 6	1031	10053	9.8
13	1056	9986	9.5
20	1376	12499	9.1
27	1033	8324	8.1
Oct. 4	1040	7587	7.3
11	790	5676	7.2
18	991	7831	7.9
25	754	6245	8.3
Nov. 1	753	5129	6.8
8	848	6810	8.0
15	712	4665	6.6
22	678	4958	7.3
29	692	4969	7.2
Dec. 6	726	5727	7.9
13	507	3450	6.8
20	591	3745	6.3
27	379	1895	5.0
Jan. 3, 1909	562	2973	5.3
10	361	1924	5.3
17	506	2605	5.1
24	604	2617	4.3
31	548	2697	4.9
Feb. 7	560	2791	5.0
14	580	2843	4.9
21	552	2548	4.6
28	555	2501	4.5
Mar. 7	766	3542	4.6
14	516	2459	4.9
21	652	2835	4.3
28	613	2447	4.0
April 4	603	1551	2.6
11	523	1437	2.7
18	531	1470	2.8
25	717	1513	2.1
May 2	590	1487	2.5
9	482	981	2.0
16	614	1525	2.5
23	455	1154	2.5
30	662	1240	1.9
June 6	681	1540	2.3
13	613	1508	2.5
20	609	1950	3.2
27	759	3878	5.1

observed. It is noteworthy that this increase coincided exactly with the commencement of the rainy season, and consequently also with the rise in humidity and with the fall in temperature. After August 30th there was again a rapid fall during the month of September to 8·1 fleas per rat in the last week of that month. In October, November and the first week of December an average of about 7·5 fleas per rat was maintained. During January, February, and March an average of about 4·5 fleas per rat was recorded. In the beginning of April a sudden drop in the number of fleas was noted to about 2·5 per rat, and this figure was maintained throughout the remainder of the hot weather and first half of June¹.

The rainy season of 1909 commenced rather earlier than usual, a few heavy thunder showers occurred towards the end of May associated with a considerable fall in the temperature. The onset of the rains therefore occurred three weeks earlier in this year than in 1908 when the monsoon was somewhat delayed. It is interesting to note that the rise in the number of fleas commenced in 1909 after the week ending May 31st, when the average number of fleas reached its lowest point, *i.e.* 1·9 per rat, while in the year 1908 the lowest figure recorded, *viz.* 1·3 per rat, was in the week ending June 21st, that is, three weeks later, corresponding exactly with the later onset of the rains in that year.

Other hosts of L. cheopis. Not many animals other than rats were examined for fleas. On about 22 squirrels which were chloroformed no *L. cheopis* were found. It is noteworthy, however, that on one of these brought in on 6th October 1908, 19 fleas closely resembling *Ceratomyllus fasciatus* were found. Although a certain number of this species were found on the rats examined by us in the Punjab, none were ever noticed amongst the rat fleas of Poona. No fleas other than *Loemopsylla cheopis* were found on mice, and of these they harboured only very few as compared with *Mus rattus*. As an example we may give the figures obtained in the month of July, when the average number of fleas per *M. rattus* was 4·4, whereas the average number per mouse (eighty-two examined) was only 0·26².

¹ These observations have been repeated in Poona for the year 1909—1910 (when there was no epidemic plague) and very similar results have been obtained.

² A rat of 100—150 grammes has nearly three times the skin area of a mouse of 20—30 grammes.

PART III.

DEDUCTIONS DRAWN FROM THE DATA COLLECTED, ESPECIALLY AS TO
THE SEASONAL PREVALENCE OF PLAGUE IN POONA.

A. The significance of imported infection.

It is an axiom to state that there can be no plague without the plague bacillus. The factors suggested by the Commission which influence the seasonal prevalence of plague can only do so in the presence of plague infection. The introduction of infection into a place is thus a matter of prime importance, and the first deductions to be made from our observations in Poona have reference to this important point.

First, the evidence we have collected shows that a plague case, imported into a place, is not necessarily associated with other cases. Thus we saw that during July and August eighteen cases of plague were imported into the city, yet the subsequent cases were not directly associated with these. Any evidence we were able to collect regarding the infectivity of plague is in conformity with the view put forward in our previous reports, that man generally acquires infection from the rat, that, in short, the epidemic is dependent on the epizootic.

Secondly, the fact that the first indigenous cases of plague in the city bore no relation to, but were usually widely separated* from, the first imported cases of the disease, suggests the possibility that infection can be brought into a place apart from persons suffering from the disease. A reasonable hypothesis to explain the carriage of infection in this way is to suppose that infected rat fleas are carried by human agency from infected to uninfected places independently of persons suffering from the disease.

Thirdly, it follows from the two deductions above that the importation of plague cases to a place is only an imperfect measure of the extent to which potential infection is being brought to it. The arrival of infected persons indicates that possibly other persons are coming from infected areas and are perhaps carrying with them infection in the form of infected rat fleas. The enormous extent to which migration from infected to healthy places takes place in India was well illustrated during our experience in Poona. We have seen that shortly after work was commenced there four cases of the disease were imported into the

municipal area from Bombay, two of the cases were actually taken out of the train ill and removed to hospital, while two others developed the disease immediately after arrival in Poona. Infection, about this time, also seems to have been carried to Kirkee, for three cases of plague were reported there in the end of May and beginning of June; previous to these cases none had occurred for more than eighteen months. The rats in the neighbourhood of the railway station were already infected before our inquiry started, or at least became infected shortly after. An epidemic and epizootic originating in this way extended rapidly, especially in Kirkee, affording a favourable opportunity for extensive importation of infection into Poona City, which, at this time, appeared to be free from infection, for the last indigenous case of plague had occurred in the end of January, and the examination of more than twenty thousand rats caught in the city failed to reveal the presence of the disease among them. The extent to which infection was imported into the city is apparent in the fact that during July and August eighteen cases of the disease were brought to it, fourteen of these coming from the suburbs, while four were from places more distant and were carried thither by rail. With the development of the epidemic in Poona City in the month of November infection must have been disseminated far and wide, for the municipal authorities estimate (and we had no reason to doubt this estimate) that from fifteen to twenty thousand people left the city for the surrounding towns and villages. Fortunately the conditions for the development of the disease had by this time become to some extent unfavourable, but, had this emigration and evacuation of the city occurred at an earlier period of the year, opportunities for the conveyance and propagation of infection would have been more favourable and a widespread epidemic might easily have occurred.

Fourthly, since the importation of infection is largely a matter of chance and may occur at any time of the year, the successful implanting of it will largely depend on whether the factors which influence the development of the disease are favourable or otherwise. The importation of infection at a season of the year when these factors are week by week becoming more favourable will not only assure a more successful implantation of it, but will also occasion a severer epidemic than had the infection been brought at a time when the favourable influences are on the wane. In Kirkee, for example, infection was successfully implanted there in June, and the epidemic, developing with a rising flea prevalence, was therefore very severe; while in Poona infection only took

root towards the end of August, when already the number of fleas was about to decline, so that the epidemic here was comparatively mild. Further evidence bearing on this point will be discussed when the influence of the number of fleas on the course of the epidemic is considered.

B. The influence of climate on plague in Poona.

In our paper on the seasonal prevalence of plague (*Journ. of Hygiene*, vol. VIII. No. 2, page 287) in discussing the influence of climate, we concluded from the evidence we had collected that a high temperature, especially when it reaches a daily mean of 85 or 90° F., or a low temperature, that is a mean below 50° F., was very unfavourable to plague. Our experience in Poona (see Chart I) showed that the influence of climate, in these directions at all events, could play only a small part in checking the plague. It was indeed for this reason that Poona was selected as a place worthy of observation, for, unlike Bombay and the Punjab villages where we had worked, epidemics came to an end here apart from the influence of temperature on them. But in the paper referred to above (p. 273) we considered that climate might influence the seasonal prevalence of plague in so far as it reacted on the life history and habits of the flea. We have shown, in the present report, that in Poona climate, especially the atmospheric humidity, plays an important rôle in causing a variation in the number of fleas found at different seasons of the year. In Chart IV the figures representing mean temperature and humidity for weekly periods during the year of our observation have been plotted against those which show the average number of fleas per rat for each week. It will there be seen how close is the relation between the percentage humidity and the flea prevalence, and how little this latter is affected by temperature. The influence of climate then on the seasonal prevalence of plague in Poona is largely exerted through its effects on the prevalence of fleas.

C. The influence of the virulence of the bacillus on plague in Poona.

No evidence was obtained in Poona to show that the virulence of the plague bacillus played any part in influencing the seasonal prevalence of plague there.

D. *The influence of (a) variations in the number of rats and (b) variations in the proportion of immune to susceptible rats on plague in Poona.*

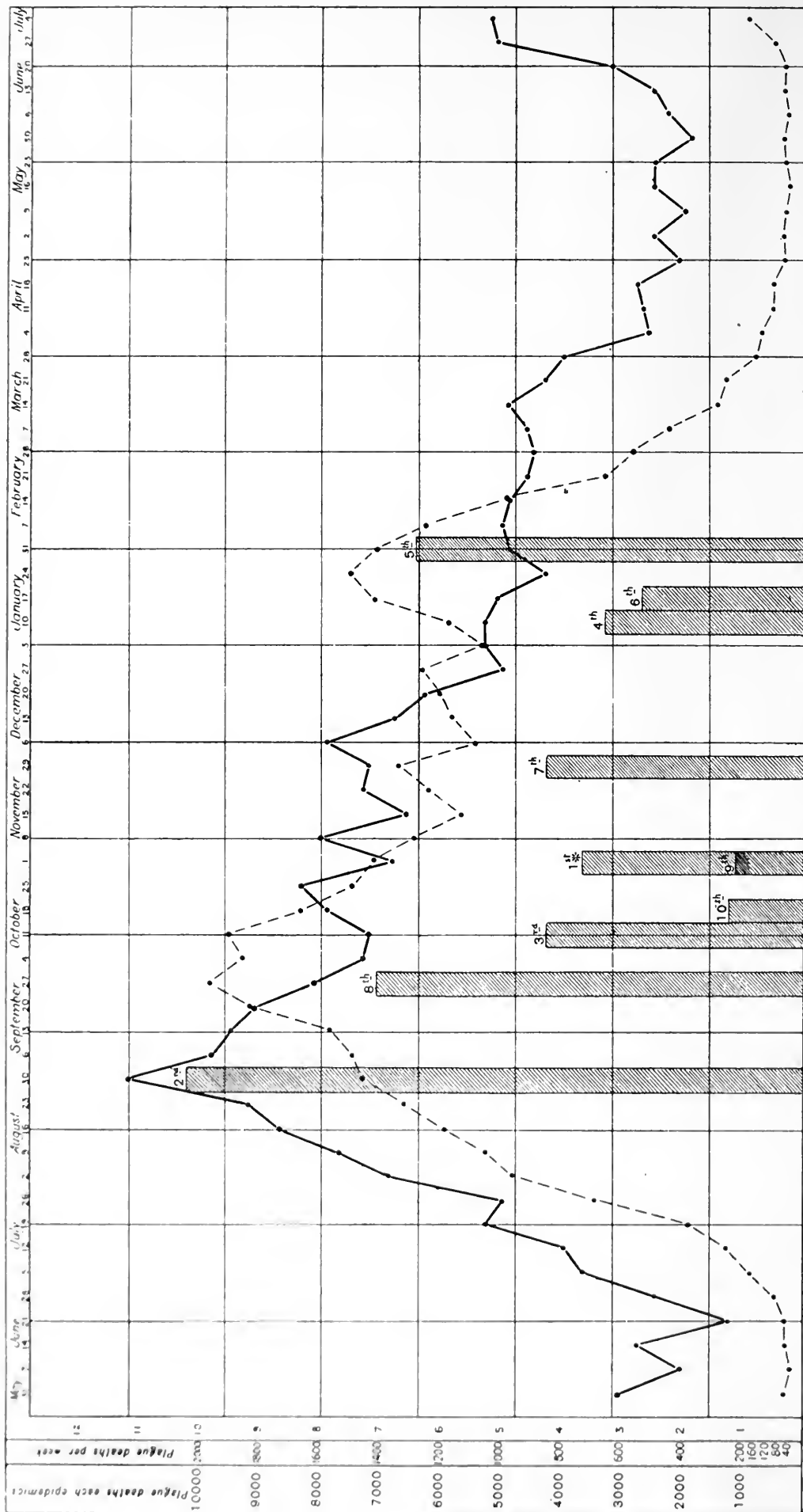
(a) Our observations showed that when plague broke out in Poona City the number of rats per house was at its maximum for the year; with the progress of the epizootic the number decreased rapidly. This diminution in the number of rats caught, however, continued after the epizootic had ceased, in spite of active breeding among the rats, possibly because the capture of rats on a considerable scale was still continued, eight thousand rats being killed in the course of the three months following the close of the epizootic. There can be little doubt that the decrease in the number of rats in Poona City with the progress of the epizootic played some part in bringing it to a close; an increase in the number of immune individuals and a decrease in the number of fleas per rat contributed to this end¹. The influence of a reduction in the number of rats, as the main, if not the only, cause leading to the termination of an epizootic, was especially well illustrated in Kirkee. Here the disease began to die out when it was springing to life in the city. We have shown that, save in respect to the number of rats present in each place, the other conditions necessary for the development of plague were favourable; thus with the decline of the epidemic in Kirkee the rats caught per 100 traps set fell from thirty-three to only nine, though at the same time the number of fleas per rat rose from approximately six to ten: the influence of climate need not be considered, for it was the same in both, the places being adjacent to one another. (b) There can be little doubt that an increase in the proportion of immune to susceptible rats as an epizootic progresses assists to some extent in bringing it to a close. In Poona no very direct evidence however was obtained in favour of this contention other than that, at the close of the epizootic in the city, an increasing number of rats were found which were either recovering or had actually recovered from the disease.

¹ The possibility must be considered that a large mortality among the rats might to some extent concentrate the fleas upon the survivors and so bring about an increase of fleas per rat. As a matter of fact in Poona fleas and rats appeared to be most abundant about the same time.

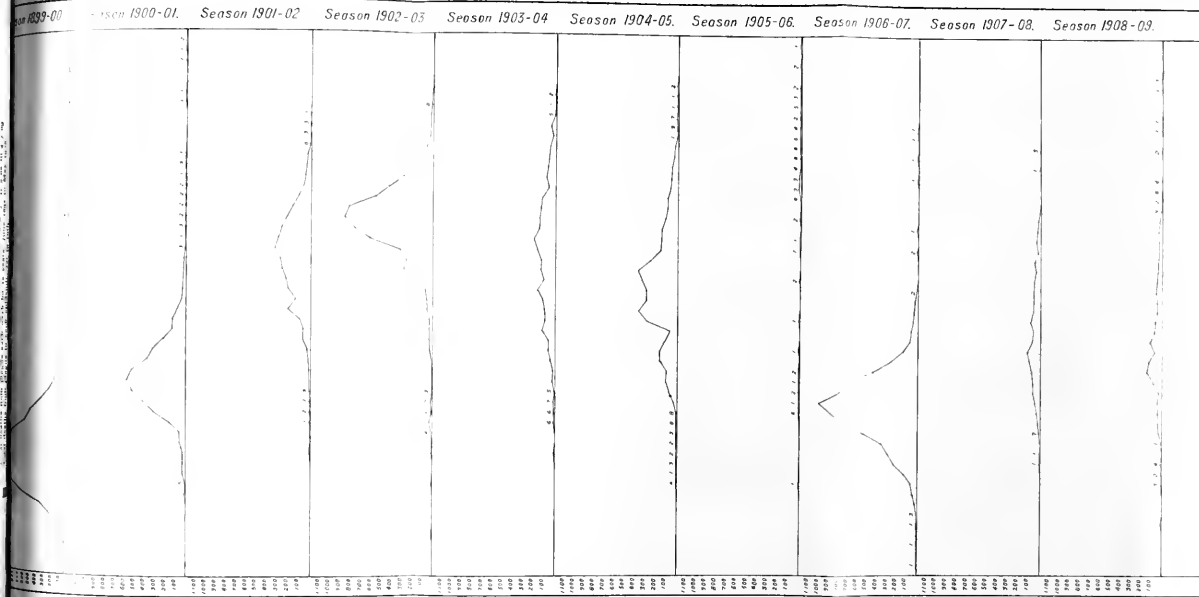
E. *The influence of variation in the number of fleas per rat on plague in Poona.*

The importance of this factor in influencing the seasonal prevalence and severity of the disease was well marked in Poona and has already been referred to above. We there drew attention to the fact that in Kirkee the epidemic, developing with a rising flea prevalence, was much more severe than the epidemic in Poona, which developed during a declining flea prevalence. On Chart VII we have plotted out in weekly figures the average number of fleas per rat. We have already noted the important bearing climate, especially the humidity, has on the prevalence of rat fleas in Poona and we have shown that the climatic conditions of the year under observation closely approximated to the mean for the ten year period 1897—1906, so that we may conclude that the variations in the flea prevalence found during the year 1908—1909 approximated very closely to the normal. On the same chart we have plotted out the figures obtained by adding together all deaths from plague which occurred within the city during each of the same weekly periods since June 1898 (the figures available for the early part of the first epidemic being somewhat inaccurate have been excluded). These figures show the extent to which plague has prevailed in each week of the year calculated on the experience of twelve years, that is, of ten epidemics. The curve obtained by plotting these figures on the chart will be seen to correspond very closely with the curve representing the flea prevalence, except for a period in January and February when the two curves slightly diverge: an explanation however for this divergence will be offered presently. In the same Chart VII each of the epidemics which have occurred in Poona City during the past twelve years has been represented in a series of shaded columns. The columns have been drawn to scale, so that each represents in height the severity of a particular epidemic as estimated by the total number of deaths from plague which have occurred during that epidemic (see Table IV p. 495). In addition the columns have been placed in the chart in the weekly period in which each epidemic reached its acme, that is, the week in each epidemic in which the maximum number of deaths was recorded. Taking for granted that the flea prevalence during each of the epidemics conformed to the experience of the year 1908—09, we may formulate the rule that the severity of the several epidemics bears a direct ratio

CHART VII



POONA



POONA

Plague deaths in Poona City (excluding all suburbs) for 13 years

100
000
500

CHART VI

1305-1



to the number of fleas per rat found at the time when each epidemic reached its acme; thus, the severest epidemic, the second, reached its acme in the last week of August, a period corresponding with the greatest flea prevalence of the year 1908—09. The second epidemic in point of severity was the eighth in numerical sequence. This epidemic reached its acme in the last week of September when the number of fleas, judging from our experience in 1908—09, was somewhat less than the number in the last week of August, but greater than the number found at the time of year when any other epidemic, except the first, reached its acme.

Certain exceptions to this rule however are to be noted, namely, the fifth epidemic as well as the ninth and tenth. Good reasons we believe can be given to explain these exceptions. Considering, first, the fifth epidemic which occurred in the year 1902—03 and which, it will be noticed, is the third epidemic in point of severity, we find that the climatic conditions at the time when this epidemic prevailed were quite exceptional and conformed in respect to humidity (and thus presumably also in respect to flea prevalence) to the conditions usually found during September, October, and November, *i.e.* the period succeeding the rains, and not to the usual conditions found in January and February, the months in which this epidemic was at its height. In Chart II the mean half monthly humidity of the year 1902—03, the year of this exceptional epidemic, is compared with the mean for the ten years 1897—1906 and with that of the year 1908—09. How materially the year 1902—03 differed from the normal, and the year of our observation, is therein manifest; attention, especially, may be drawn to the records of December and January, the figures 81 and 76, 71 and 70% humidity for each half of these two months of the year 1902—03, contrasting with 55 and 56, 57 and 61% as a mean record for the same half monthly periods during ten years. It is evident, that in December and January 1902—03, the climatic conditions in point of humidity approximated to that found during the period immediately succeeding the rains, and it is reasonable to suppose that the flea prevalence too in this year approximated to that usually found at this season. If this were the case the unusual severity of the fifth epidemic would be explained, and it would naturally be placed between the eighth and the third in numerical sequence, making it thus the third in point of severity. In the advent of this unusual epidemic we have also an explanation for the want of correlation between the flea prevalence curve and the plague curve above referred to in January and February.

Passing to the exceptions noted in the ninth and tenth epidemics we have already drawn attention to the elaborate arrangements for the suppression of plague which were adopted by the committee appointed by Government during the ninth and tenth epidemics; especially noteworthy figures have been given to show the larger extent to which preventive inoculation was practised during these years, and the estimate of the municipal authorities has been quoted to show the enormous extent to which evacuation was resorted to in the tenth epidemic (see p. 497). Moreover our experience during this epidemic showed that these measures had a marked effect in checking the epidemic, for, contrary to our past experience in Bombay and in the Punjab, the epidemic of 1908—09 reached its acme before the epizootic¹: had these measures not been in force, we have reason to think that the epidemic might have increased in severity till the month of December, when the epizootic was at its acme, in place of declining, as it did, early in November.

Having thus disposed of the exceptions to the rule formulated above (exceptions which may be said to prove the rule), that the severity of the several epidemics in Poona bear a direct relation to the number of fleas present on rats at the time each epidemic reaches its acme, we have established the fact that, in Poona, the factor which has the greatest influence on the seasonal prevalence of plague is the flea infestation of the rats, and this, again, is largely influenced by the climatic conditions, especially the atmospheric humidity.

PART IV.

CONCLUSIONS.

1. Plague may occur in Poona at any time of the year, but the disease generally prevails in well-marked epidemics.
2. The period of the year at which an epidemic may occur depends largely on the time at which infection is successfully implanted.
3. When infection takes root immediately before the flea season or with a rising flea prevalence a severe epidemic follows.
4. When infection takes root immediately after the flea season or with a declining flea prevalence a less severe epidemic results.

¹ The failure to obtain dead rats in any quantity in Poona may have given an erroneous picture of the intensity of the epizootic at different times.

5. The severity of an epidemic in Poona thus bears a direct ratio to the flea prevalence at the time it reaches its acme.

6. The flea prevalence in Poona is intimately connected with the climatic conditions, especially with atmospheric humidity.

7. Other conditions being favourable, an epidemic may come to an end on account of the scarcity of rats.

8. The epidemics in Poona City, however, generally terminate owing to a combination of adverse factors, viz. a decrease in the number of fleas, a decrease in the number of rats, and an increase in the proportion of immune to susceptible rats.

9. Which of these three factors will exercise the preponderating influence in terminating an epidemic will depend on the season of the year at which the epidemic occurs, the two latter probably exercising the greater influence during the rains and the period immediately succeeding the rains, when the flea prevalence is high, while the former will have the greater effect during the cold and hot weather when the flea prevalence is low.

10. The climatic conditions in Poona affect the course of plague mainly through their effect on flea multiplication.

11. No evidence could be obtained to show that the virulence of the bacillus plays any part in terminating an epidemic.

12. The preventive measures adopted in the last two epidemics seem to have materially decreased the mortality from plague.

XXXVIII. FIRST REPORT ON INVESTIGATIONS INTO
PLAGUE VACCINES.By SYDNEY ROWLAND, M.A., M.R.C.S.,¹*Of the Lister Institute.*

I. INTRODUCTION.

THE primary source from which all vaccines are derived is a culture of the organism against infection by which it is desired to protect the animal. The culture may be used as a vaccine either alive or dead. We are concerned here only with the latter. Such a killed culture comprises the organism itself and the products of its metabolism, together with part or the whole of the culture medium in which it has grown. This mixture may be called a *whole vaccine*. By appropriate treatment a "whole vaccine" may be deprived of some or all of such constituents as are not concerned in bringing about, when inoculated into animals, those changes which constitute protection. Such a vaccine may be called a *derived vaccine* in the case when all the immaterial constituents are not removed, the term *antigen* being reserved to designate that substance which alone is concerned in conferring protection. The main object of this investigation is the preparation of a derived vaccine with the hope that the antigen may ultimately be isolated.

Whole vaccines may be prepared in any convenient way provided the contained antigen is not injured. Derived vaccines are prepared by a process of trial and error, using a whole vaccine as the material of investigation. Each preparation must be tested for antigen content by

¹ For a brief period at the beginning of the investigation, the late Dr T. Carlyle Parkinson was associated with Mr Rowland in this investigation. His untimely death from pneumonic plague on February 4th, 1909, clouded the early months of the work. By the loss of Dr Parkinson the Commission has been deprived of the services of an enthusiastic and most promising investigator.

some such method as is described in this report. A continuation of the processes of elimination which converted a whole vaccine into a derived vaccine, if carried sufficiently far, will lead ultimately to the isolation of the antigen.

II. SUMMARY OF PREVIOUS WORK.

That it is possible to immunise an animal against the subsequent inoculation of a living virulent plague culture was first shown by Yersin, Calmette and Borrel¹, who employed a suspension of plague bacilli killed by heating to 50° C. for one hour. Kolle² subsequently employed suspensions heated to 65° C. for several hours, while Wyssokowitz and Zabolotny³ used suspensions heated to 60° C. Haffkine⁴ employed heated broth cultures which he grew for some time, believing that some substance was gradually produced by the organisms which went into solution in the broth, and that this substance contributed to the immunising process. Gabritschewsky⁵, abandoning the method of killing by heat, employed cultures that had been killed by glycerin, but soon became involved in difficulties due to the poisonous action of the glycerin when injected subcutaneously. The German Plague Commission⁶ returned to the use of cultures killed by heat with the extra precaution of the addition of a small proportion of carbolic acid to the vaccine. They observed that if the carbolic was added before the organisms were killed by heat, the immunising value of the vaccine was less than it was when the organisms were heated first and the carbolic added afterwards. They also observed that it was advisable to employ as low a temperature as possible to kill the bacteria, and that at a sufficiently high temperature the immunising value was completely lost. Calmette⁷ employed bacilli that had been killed by drying in a desiccator. He grew his bacilli either in broth and obtained them by

¹ Yersin, Calmette and Borrel, *Ann. de l'Inst. Pasteur*, 1897.

² Kolle, *Deutsche Med. Woch.*, 1897.

³ Wyssokowitz and Zabolotny, *Ann. de l'Inst. Pasteur*, 1897.

⁴ Haffkine, *Indian Med. Gaz.*, 1897; *Ref. nach Schottelius, Hygien. Rundschau*, 1901, p. 3 ff.

⁵ Gabritschewsky, *Ref. Centralbl. f. Bakt.* 1898; Russisches, *Archiv f. Pathol. klin. Med. u. Bakt.*, 1897.

⁶ Gaffky, Pfeiffer, Sticker und Dieudonne, *Arbeiten aus dem Kaiserlich. Gesundheitsamt*, 1899, Bd. xvi.

⁷ Cited by Tavel, Krumbein u. Glucksmau, *Zeits. f. Hygiene*, 1902, Bd. xL. p. 239.

filtration, or on the surface of agar and obtained them by scraping the surface of the agar.

All these vaccines belong to the class of whole vaccines, inasmuch as they consist of the organism together with the culture medium on which it was grown and the products of the metabolism of the organism dissolved in the medium. In the case of Calmette's vaccine some of the products of growth and some of the culture medium were removed.

Strong¹ used avirulent living cultures as vaccines with some success, but as this line of inquiry does not come within the scope of the investigation dealt with in this report no further allusion will be made to it.

As to the vaccinating value of derived vaccines a considerable amount of information exists. Lustig and Galeotti² suspended the surface growth from agar in 0.75% NaOH solution, and from the extract thus obtained precipitated by means of acetic acid a flocculent white substance which was of some vaccinating value. This substance could either be dissolved in weak alkalis and used at once, or dried: in the latter form it kept well. 0.36 mg. of this substance immunised a rat and no unfavourable symptoms followed its subcutaneous inoculation. To these authors belong the credit of first producing a vaccinating substance which could be accurately graduated, a great step in advance. Lustig and Galeotti considered their substance to be a nucleo proteid. Tavel, Krumbein and Glucksman³ grew broth cultures by Haffkine's method and precipitated them, after a month's growth, by ammonium sulphate. The precipitate was extracted with 1% soda, and acetic acid was used to throw down from this extract what the author considered was a nucleo proteid. This was dried in vacuo and preserved in the form of a powder. For use it was dissolved in 1% sodium carbonate.

Another vaccine was recommended by Terni and Bandi⁴. It consisted of a culture of the plague bacillus grown not on an artificial medium, but in the peritoneal cavity of living guinea-pigs. As in the case of other whole vaccines the organisms were killed by heat and the extra precaution was taken of adding a small percentage of carbolic acid.

Another method in which the organisms are grown in the living animal has been proposed by Hueppe and Kikuchi⁵. Under these

¹ Strong, *Philippine Journ. of Science*, 1906, p. 182, and 1907, p. 155.

² Lustig and Galeotti, *Deutsche Med. Woch.*, 1897, pp. 23, 227 and 289.

³ *Zeitsch. f. Hyg. u. Infektionskrankh.*, Leipzig, 1902, xl. p. 239.

⁴ Terni and Bandi, *Ibid.*, 1900, xxvi. p. 463; *Rev. d. Hyg.*, Paris, 1900, xxii. p. 62.

⁵ Hueppe and Kikuchi, *Cent. f. Bakt. Orig.*, 1905, l. p. 519.

circumstances a class of bodies known as aggressines are produced, which are capable of being employed as vaccinating substances. An investigation of this question will form the subject of a further report.

Still another method depending on the growth of the organism in the living body has been proposed. Klein¹ grows his organisms in the bodies of living guinea-pigs, removes from the dead animals the buboes, spleen, lungs and liver, minces them finely, and dries the minced material in thin layers over sulphuric acid. This treatment kills all the plague bacilli. The dose of this material which he considers contains the specific toxin of the plague bacillus together with "other substances of unknown nature and action" is determined by weight, the immunising dose for a rat being from 10 to 15 mg. S. Wallannah² also proposed a similar method.

In addition to the methods of immunising by means of a vaccine alone—however prepared—methods have been proposed which combine the use of a vaccine with that of plague immune serum, that is to say, serum taken from an animal that has previously been inoculated with some preparation of the plague bacillus or even with the living bacillus. Thus Shiga³ employed a mixture of immune serum and an emulsion of plague bacilli which had been killed by heat, and Besredka⁴ employed a similar mixture but heated his bacilli after the addition of the serum (which addition had caused them to clump together). The mass of organisms which he obtained in this way was washed, made into an emulsion, and used as a vaccine. Gosio⁵ made use of the precipitating action of immune serum in the preparation of vaccine on a large scale.

In a later method of Besredka⁶ the use of immune serum was abandoned in favour of normal horse serum, organisms which have been treated (after having been killed) with normal serum being stated to be nontoxic. In a still later method Besredka⁷ starts with an emulsion in salt solution of a 48-hours' growth on agar. The organisms are killed by heating to 60° C. and dried in vacuo. The dry mass of organisms is now ground in an agate mortar with salt and the ground

¹ Klein, *Rep. to Loc. Gov. Board on a new Plague prophylactic*, London, 1906; *Brit. Med. Journal*, 1906, p. 155.

² Wallannah, *Ann. de l'Inst. Pasteur*, 1905, ix. p. 589.

³ Shiga, *Ber. über die Pest in Kobe und Osaka*, Tokio, 1900, p. 54.

⁴ Besredka, *Ann. de l'Inst. Pasteur*, 1902, xvi. p. 918.

⁵ Gosio, *Zeitsch. f. Hyg. u. Infektionskrankh.*, Leipzig, 1905, L. p. 519.

⁶ Besredka, *Ann. de l'Inst. Pasteur*, 1905, xix. p. 479.

⁷ Besredka, *Ibid.*, 1906, xx. p. 304.

mass made into an emulsion in water. After shaking, standing and centrifuging, a solution is obtained which he considers contains the endotoxine of the plague bacillus.

Some successful attempts have been made to confer immunity by the use of a toxin which under certain circumstances is excreted by the plague bacillus, but as the circumstances which determine this secretion are altogether unknown but little use has been made of it. With our present knowledge, the appearance of a toxin in a fluid culture of the plague bacillus is altogether fortuitous, some cultures will contain it, and others grown under apparently identical conditions will be without any toxic action. Markl¹ obtained some success using this method. He grew his cultures in broth to which a small quantity of serum had been added. He obtained evidence of the existence of toxic substances in solution, especially in old cultures, and considered that a method of immunising which employed both bacilli and free toxin as the vaccinating material was to be preferred, as it rendered the animals (in his opinion) resistant not only to the invasion of living organisms, but also to the toxin produced by them. Dean² also obtained evidence of the existence of free toxin in solution in the case of old cultures, and showed that it could be separated from the organisms by filtration. Further reference will be made to this work when the question of antitoxic serum is considered.

A consideration of the work of these various authors suggests that

1. The plague bacillus contains a toxin within its cell substance.
2. The plague bacillus contains a nucleo proteid within its cell substance.
3. That occasionally some amount of toxin becomes free in the culture medium, but that the conditions which determine this are not clearly defined.

III. GENERAL METHODS OF PREPARATION OF VACCINES.

It is an axiom that an ideally perfect vaccine is a solution of the antigen in some inert fluid. Starting with a culture of the plague organism, any treatment that we may subject it to in the quest of this perfection must fulfil the following conditions:

¹ Markl, *Ann. de l'Inst. Pasteur*, 1898, xxiv.; *Wien. Med. Woch.*, 1900; *Zeit. für Hyg.*, 1901, xxxvii.

² Dean, *Studies in Pathology in celebration of the Quater Centenary of the Univ. Aberdeen*, Aberdeen, 1906.

1. The organism must be killed.
2. The antigen must be injured to the minimal extent.
3. Substances other than antigen must be removed as far as possible.

The methods that are available for killing the organism are restricted to those which do not greatly injure the antigen. Such methods are much fewer than might be supposed. Thus heat, the most convenient of all methods, must be used with caution, for in the case of every antigen that has yet been investigated, heating in the presence of water has a deleterious action. The usual means of minimising this destructive action of hot water is to employ as low a temperature as possible and to repeat the application on several occasions. Speaking generally heat is an agent that is better avoided if possible.

The objections that can be urged against the use of heat as a killing agent apply to most of the chemical means available. Even a comparatively innocent antiseptic may entirely destroy the antigen. Chloroform and toluol do not seem to appreciably damage it, whereas cymol destroys it. (See p. 564.)

There remain certain mechanical and physico-chemical methods which while destroying the life of the bacillus may not greatly injure the antigen:

1. mechanical disruption of the bacilli by grinding; and
2. desiccation.

The present writer in conjunction with the late Dr. A. Macfadyen¹ devised a purely mechanical method of killing the organisms which had the advantage of being conducted at a low temperature, -180°C . The bacilli were brittle and could be ground to a fine state of division at this low temperature, thus facilitating the subsequent solution of the contained substances.

Desiccation of cultures over calcium chloride or sulphuric acid has been used to kill plague bacilli by Calmette and Klein. It is an uncertain process and difficult to accomplish satisfactorily unless a very thin layer is exposed. I have found it unhandy in operating upon large quantities of bacilli and not free from danger. To obviate these disadvantages I have, instead of drying by exposure to dry air in thin layers, mixed the bacterial paste with a suitable amount of anhydrous sulphate of soda. This works very satisfactorily. The hard mass formed can be melted

¹ Macfadyen and Rowland, *Ber. der Deut. chem. Ges.* 1900, xxiii; *Proc. Roy. Soc.*, London, LXXI. p. 77; *Cent. für Bact.*, I., xxxv. p. 415.

above 35° C. and the bacteria were always found to be killed after desiccation in this manner.

The early experiments to be shortly detailed were conducted with plague bacilli killed in this way. Subsequently it was found that preliminary killing with chloroform vapour did not materially influence the yield of antigen whilst considerably reducing the danger of the various manipulations.

IV. METHODS EMPLOYED IN DETERMINING THE DEGREE OF IMMUNITY CONFERRED AND IN INTERPRETING THE EXPERIMENTAL RESULTS.

In an investigation of this kind, which aims at giving quantitatively the comparative value of various toxic and immunising substances derived from the plague bacillus, difficulties are met with, for not only is there a variable resistance amongst the test animals employed, but in addition the virulence of the test culture employed may also vary. We require to be assured that during the progress of the work both these factors have remained reasonably constant.

As a preliminary, a culture was selected from a large number received from Bombay which, when grown on agar at laboratory temperature, appeared to retain a uniform virulence for many successive generations. It has been kept on agar, at the temperature of the laboratory, in the dark. For the purposes of a test it is grown in rat broth, in which medium the plague bacillus grows well without excessive clumping, and as a further precaution some glass beads are included in the culture flask with which clumps can be broken up. Finally, in order to ensure as homogeneous a suspension as possible, the culture is filtered through cotton wool immediately before using. As a test dose $\frac{1}{10}$ c.c. of a 72-hours' broth culture of this organism injected subcutaneously has been employed. This dose does not kill every rat injected with it, and it was found that multiplying the dose by ten or reducing it to $\frac{1}{100}$ c.c. did not greatly influence the proportion destroyed by it. (See Table I.)

Method of inoculation. Many observers have used the cutaneous method of inoculating (*i.e.* rubbing a culture into a portion of recently shaved skin) rather than the sub-cutaneous, but as experiments made on this point led to the conclusion that the sub-cutaneous method was more constant in its results, it was accordingly adopted.

TABLE I.

Strain	Dose c.c.	Rat	Result
No. 1	{ 1·0	(1	+
	{ 1·0	(2	0
	{ 0·1	(3	+
	{ 0·1	(4	+
	{ 0·01	(5	+
	{ 0·01	(6	0
	{ 0·001	(7	+
	{ 0·001	(8	0
No. 2	{ 1·0	(1	+
	{ 1·0	(2	+
	{ 0·1	(3	+
	{ 0·1	(4	0
	{ 0·01	(5	0
	{ 0·01	(6	0
	{ 0·001	(7	+
	{ 0·001	(8	0
No. 3	{ 1·0	(1	+
	{ 1·0	(2	+
	{ 0·1	(3	0
	{ 0·1	(4	0
	{ 0·01	(5	+
	{ 0·01	(6	0
	{ 0·001	(7	+
	{ 0·001	(8	0

+

= animal died.

0

= animal survived.

It was then necessary to determine—using a large number of animals—how many rats this culture would kill. One hundred rats, taken at random out of the stock, were inoculated with the dose of culture mentioned above. Of this 100, 22 survived.

TABLE II.

Inoculation of 100 rats for statistical error.

		+ = died of plague.		0 = recovered.			
Rat	Result	Rat	Result	Rat	Result	Rat	Result
1	+	26	+	51	+	76	0
2	+	27	+	52	0	77	+
3	+	28	+	53	+	78	+
4	+	29	+	54	+	79	+
5	+	30	0	55	+	80	+
6	+	31	+	56	+	81	+
7	+	32	+	57	0	82	+
8	+	33	+	58	0	83	+
9	+	34	+	59	+	84	0
10	0	35	+	60	+	85	+
11	+	36	0	61	+	86	+
12	+	37	+	62	+	87	+
13	+	38	+	63	+	88	0
14	0	39	+	64	0	89	0
15	+	40	+	65	+	90	+
16	0	41	+	66	0	91	+
17	+	42	+	67	+	92	0
18	0	43	+	68	0	93	+
19	+	44	+	69	+	94	+
20	+	45	+	70	0	95	+
21	+	46	0	71	+	96	+
22	0	47	+	72	0	97	+
23	+	48	0	73	+	98	+
24	+	49	+	74	+	99	+
25	+	50	0	75	+	100	+

Having in this experiment used a large number of rats the chances are some twenty to one that we should not get a lower percentage mortality than 70 or a higher mortality than 86 in the population from which the sample 100 was drawn.

It would obviously be inconvenient to have to repeat this control series of 100 rats every time an experiment was made, but a small test series of ten rats could be and was employed on every occasion.

The next question arises, How many of a test series of 10 should die to enable it to be said that neither the natural immunity of the rats

used nor the virulence of the culture has varied from that found previously in the series of 100 ? This is a statistical problem of greater complexity than I am capable of dealing with, but my colleague Mr Greenwood, Statistician to the Lister Institute, has been good enough to come to my assistance, and I wish here to express to him my gratitude. Mr Greenwood informs me that the question may be answered in the following way :

If p be the number of deaths and n the whole number of animals used in the first experiment, if $q = n - p$, $\bar{p} = \frac{p}{n}$ and $\bar{q} = \frac{q}{n}$, then the most probable number of deaths to occur in a second sample of m rats is¹

$$m\bar{p} + \frac{m}{n+2} (\bar{q} - \bar{p}).$$

Using this formula we conclude that on the basis of a preliminary sample of 100 having given 78 deaths, we should expect a subsequent random sample of 10 to yield 7.745 deaths. Now we have 19 such samples of ten controls each, and we require to know how their yields compare with the expectation. The mortality in these controls is shown in Table III below :

TABLE III.

Date	Rats		Date	Rats	
	Survived	Died of plague		Survived	Died of plague
March 24	1	9	July 29	2	8
31	2	8	Oct. 8	4	6
April 5	0	10	18	2	8
21	3	7	29	5	5
May 26	1	9	Nov. 11	3	7
June 5	0	10	23	1	9
19	3	7	30	0	10
July 9	1	9	Dec. 15	5	5
17	0	10	Jan. 21	3	7
25	1	9		37	153

The approved way of testing agreement² is to form the sum of $\frac{(\text{Expected number} - \text{Actual number})^2}{\text{Expected number}}$ for the whole number of trials and to compare the sum with its tabled values.

Applying this test we have χ^2 (the sum in question) = 6.294 and looking up the table we have $P = .994$. Which means that if 7.745 is

¹ Pearson, *Phil. Mag.*, 1907, pp. 365—378.
² Pearson, *Ibid.*, 1900, pp. 157—175 ; Elderton, *Biometrika*, i. p. 155. 1902.

really the most probable value, we should, owing to the unavoidable errors of random sampling, get no better agreement than actually found 99 times in every 100 repetitions of the series.

Some other tests could be and have been employed by Mr Greenwood, who is responsible for the previous calculation. The result of these analyses is, in his opinion, sufficiently definite to support the following statement :

The variations in the mortality among the controls are perfectly consistent with the results yielded by the preliminary inoculation of 100 rats. They do not need to be explained by (1) a change in the natural immunity of rats, or (2) any alterations in the virulence of the culture. This is a most important and satisfactory assurance.

We have now to consider what amount of diminution in mortality must occur in the groups of rats injected with various products of the plague bacillus before such fall in mortality can be safely regarded as causally related to the treatment they have received ; in other words, it is necessary to ascertain what chance variation may be reasonably expected in groups of the size employed—15 to 50 animals.

Having ascertained the mortality (p) in a sample of n individuals (in our case 100) the probable mortality in a second series of m individuals can be calculated¹ from the formula

$$p \frac{m}{n} \pm \cdot 6745 m \sqrt{\frac{p}{n} \times \frac{n-p}{n} \left(\frac{1}{m} + \frac{1}{n} \right)}.$$

The fraction preceded by the signs \pm is called the probable error of the calculated value. The chances are equal that the number will fall within or without this range. An allowance of merely the probable error does not however afford any security and it is advisable to have as margin some multiple of this.

The chances against a mere accidental variation accounting for an unusual mortality in the second sample rapidly grows as the range allowed is increased, as may be seen from the following approximate table :

			Chances against deviation occurring ²
Margin of probable error	2 to 1
„ twice probable error	10 to 1
„ three times probable error	43 to 1
„ four times „ „	285 to 1

¹ Todhunter, *History of the Theory of Probability*, Chapter on Laplace; Pearson, 1907, *op. cit.*

² These are the chances against a deviation occurring in a given direction ; the chance against, *e.g.* a positive deviation of twice the probable error or more is 10 to 1 ; the chance against a deviation of this order, but either positive or negative, is 4.5 to 1.

Three times the probable error is usually regarded as safe, and in the immunisation experiments detailed below after setting out the mortality which actually occurred in the different series of treated animals, an additional column has been added giving the mortality which might be expected to occur once in 44 times calculated on the mortality amongst the 100 controls, by mere chance distribution of extra resistant rats. As will be seen, the mortality in the different series treated either with a vaccine composed of the whole bacillus or of one particular constituent of the bacillus reduced the mortality considerably beyond the margin allowed on the above basis. Moreover, as the experience of seven successive series of experiments was uniform, the probability against any chance distribution becomes enormously increased and the results can be relied upon as bearing with certainty the interpretation placed upon them, viz., that the major part of the diminished mortality is accounted for by the prophylactic injections.

V. PREPARATION AND INVESTIGATION AS TO CHEMICAL AND ANTIGENIC PROPERTIES OF PRODUCTS OBTAINED FROM THE PLAGUE BACILLUS.

The object of the research being to ascertain the best method of preparing an immunisator against plague, it was considered that the most likely means to arrive at this goal would be to ascertain if possible to which constituent or constituents of the bacillus the antigenic property was due, and to isolate as far as might be practicable, the active constituent. If this could be accomplished it would then be possible to investigate the development of immunity and to study the effect of varying conditions upon the more or less isolated antigen with greater exactitude. Having arrived at this knowledge one would be in a position to devise the best procedure for its extraction from the bacillus with a minimum of damage and to decide the most satisfactory course to pursue in the practical immunisation against plague.

At the outset, however, one is confronted with the problem of killing the bacilli without undue destruction of antigen. For the first stage of this investigation it is not essential that the method should be that least prejudicial to the antigen. It is however essential to choose one which leaves a considerable amount of antigen undestroyed and one which is convenient and yields consistent results. After some pre-

liminary trials, killing by chloroform was selected as satisfactorily fulfilling these conditions¹.

An attempt has been made in this investigation to employ quantitative methods. Thus it will be noted that the dose of organisms or product therefrom used in any of the experiments is expressed in terms of weight. The weight given—in milligrammes—is the weight of dry substance contained in the dose given. In the case of organisms this is determined by centrifuging a known volume of emulsion, washing the deposited organisms, drying in the oven at 105° C. and weighing the residue. In the case of a protein solution, the weight is the dry weight of the protein in solution, after precipitation and washing free from salts and non-precipitable substances.

The additional labour involved in employing these methods is considerable, but the advantages are well worth it. Such units as a "loopful," "half an agar slope," are far from satisfactory. By substituting milligrammes in the dry condition we secure the following advantages:

- (1) the possibility of repeating an experiment under the original conditions;
- (2) the ability to compare results obtained at one period of the work with those obtained at another; and
- (3) the power of selecting from a number of different preparations of the organism the one which exhibits in the greatest degree the properties we are in search of.

Another method that was found invaluable in the course of the work was the determination of the total nitrogen content of any preparation (by Kjeldahl's method). In attempting to resolve into products a material of the complexity of a bacterial body, the distribution of the nitrogen before and after the use of any solution, extraction or disintegration method often throws considerable light on what is happening.

Choice of experimental animals. The susceptibility of animals to plague infection varies considerably. Of laboratory animals the rat, mouse and guinea-pig are most easily infected, the rabbit less so. The facility with which these animals can be protected against infection also varies. Thus Yersin, Calmette and Borrel² succeeded in immunising

¹ The statement by Pick (Kraus and Levaditi, *Handbuch der Technik und Methodik der Immunitätsforschung*, Bd. 1. p. 353) to the effect that chloroform destroyed the antigen of the plague bacillus and was therefore not available for the preparation of a vaccine has not been confirmed.

² Yersin, Calmette and Borrel, *Ann. de l'Inst. Pasteur*, 1895.

rabbits but not guinea-pigs. Wyssokowitz and Zabolotny¹ immunised monkeys, and Haffkine's fluid can immunise man. The German Plague Commission² employed monkeys and rats as experimental animals and succeeded in immunising both.

The most difficult animal to immunise is the guinea-pig, and success has only followed in the case of those observers who have employed the method of living cultures. Chief amongst such is Strong³, who has succeeded with these animals and with man. MacConkey⁴ has shown that guinea-pigs and rats may be protected against plague by inoculation with the bacillus of pseudo-tubercle of rodents.

The choice of animal for the purposes of this investigation, bearing in mind the results obtained by the observers just quoted, fell on rats, and, for the purposes of the first experiments, it was decided to restrict the observations to the effects of a single inoculation, followed fourteen days later by a test inoculation of living virulent organisms. It was also thought best to employ as the index of immunity the survival or death of the animal, and to leave till later the investigation of the mechanism of the immunity conferred.

There are additional reasons for using rats as the test animals. In both man and rats the effects of the disease are explicable on the assumption of a considerable degree of susceptibility to a toxin formed from the bacillus. Thus in both animals death often occurs whilst the bacilli are still confined, or almost confined, to the lymphatic glands, that is to say, before a general septicaemia has arisen. Before the advent of death there are abundant evidences of a profound intoxication, chief amongst which is a pronounced cardiac failure. If a rat, in the earlier stages of the disease, be roughly handled, and so provoked to struggle, sudden collapse and death will often follow; the same phenomenon is often observed in man. There are thus great resemblances between plague stricken men and rats. For all these reasons rats appeared the most suitable animal on which to work out the protective power of the various bacterial products investigated.

The possibility that the toxic substances to which rats are sensitive may not be equally reactive with other animals has not been lost sight of. The results obtained and described in this report apply only to rats, and are now being extended to guinea-pigs and rabbits.

¹ Wyssokowitz and Zabolotny, *Ann. de l'Inst. Pasteur*, 1897.

² German Plague Commission, *loc. cit.*

³ Strong, *Philippine Journ. of Science*, 1907, II. 3.

⁴ *Journal of Hygiene*, VIII. (1908), 335.

Culture Medium. For the purposes of this investigation, in which large quantities of organisms as free as possible from the products of metabolism were required for chemical examination, a solid medium was essential. The quantity of growth obtained on a solid medium is much greater than that obtained in a liquid one. The question of the advantages gained by the addition of other substances to the ordinary media was not investigated, as it was found that quite a sufficient growth was obtained on a medium having the following composition: 1% Lemco, 2% peptone, 3% agar. The choice of Lemco in place of beef broth was determined by the possibilities it afforded of securing a medium of constant composition. The use of Roux flasks offers many advantages over plate or dish methods of propagating.

Substance A.

After 4 days' incubation at 32° C. the bacilli were scraped from the surface of the agar by means of a glass rod and suspended in salt solution; the emulsion of living organisms thus obtained was washed in salt solution to remove culture fluid and products of growth, and suspended in salt solution. The nitrogen in a given volume of the resulting emulsion was determined by Kjeldahl's method. The organisms were then killed by chloroform and allowed to soak in chloroform water for 2 hours at 18° C. At the end of this time the emulsion was centrifuged and the clear fluid examined. It contained proteid in solution, and a determination of the nitrogen in the fluid showed that 29.5% of the nitrogen in the emulsion had gone into solution. On adding acetic acid and boiling a precipitate was obtained, and it was found that this precipitate accounted for 17.6% of the emulsion nitrogen, the balance of 11.9% not being precipitated. On adding tannic acid a precipitate was obtained and it was found that the non-precipitable nitrogen accounted for 11.8% of the emulsion nitrogen.

Schematically the distribution of the nitrogen in a chloroform emulsion may be represented:

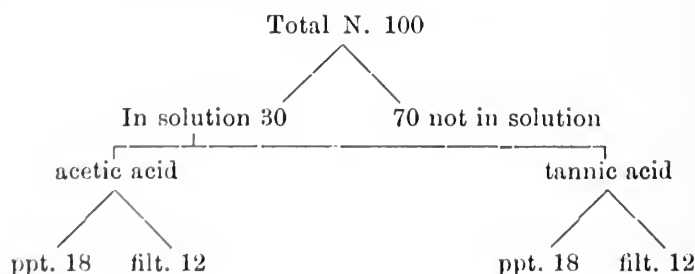


TABLE IV.

Chloroform killed. Water extracted.

Dose mg.	Rat	Result*
1·4	{ 1	+ 1 day
	{ 2	+ 1 day
0·14	{ 3	0
	{ 4	0

Chloroform killed. Extracted with dilute sodium sulphate solution. Density 1046.

2·0	{ 1	+ 2 days
	{ 2	+ 2 days
1·0	{ 3	0
	{ 4	0
0·5	{ 5	+ 2 days
	{ 6	+ 2 days

Chloroform killed. Extracted with dilute solution sodium sulphate. Density 1051.

1·0	{ 1	0
	{ 2	0
0·5	{ 3	0
	{ 4	0

Toluol killed. Extracted with dilute NaCl.

1·0	{ 1	+ 1 day
	{ 2	+ 2 days
0·5	{ 3	+ 3 days
	{ 4	0

Toluol killed. Extracted with dilute sodium sulphate. Density 1051.

1·0	{ 1	+ 1 day
	{ 2	+ 1 day
0·5	{ 3	+ 1 day
	{ 4	+ 1 day
0·25	{ 5	+ 1 day
	{ 6	+ 2 days

When precipitated from its solutions by acetic acid and re-dissolved in dilute alkali the toxicity was :

2·5	{ 1	+ 1 day
	{ 2	+ 3 days
0·84	{ 3	+ 3 days
	{ 4	0
0·42	{ 5	+ 2 days
	{ 6	+ 2 days

* Henceforth the sign + signifies died and 0 survived.

This proteid substance which goes into solution on the death of the bacillus by chloroform accounts, therefore, for 18% of the total nitrogen of the organism. It is precipitated from its solution by acetic acid. The precipitate re-dissolves in dilute alkali with facility. A solution gives Mollisch's as well as the tryptophane, biuret, and xanthoproteic reactions. It contains much combined phosphorus, and 16 to 18% nitrogen. It corresponds to a nucleo-protein, and is here called "*Substance A*."

Inoculated into rats it is toxic, but its toxicity is very variable, the lethal dose of different preparations varying from .25 to 2 mg. Table IV gives some idea of this variation. The organisms were killed with chloroform and in two cases with toluol. Dilute sodium sulphate or salt solutions were used for extraction of the substance A. The variation in the relation of nucleo-protein content to toxicity suggests that this protein is not the toxic substance, but that the latter is closely associated with it.

The association of the toxic substance with the nucleo-proteid appears to be a close one.

Immunising Properties. Of 49 rats inoculated with .01 mg. of the nucleo-proteid of solution A 34 succumbed to a subsequent inoculation of living plague bacilli (mortality 70%).

Of 30 rats inoculated with .01 mg. of another preparation of the nucleo-proteid A precipitated by acetic acid and dissolved in weak alkali 20 succumbed to the subsequent inoculation (mortality 51%).

Of 43 rats inoculated with .001 mg. of the same material 29 succumbed to the subsequent inoculation (mortality 68%).

Allowing three times the probable error, in accordance with the principles discussed on p. 546 above, a mortality as low as 64, 61 and 62 respectively might be expected to occur once in 44 times. Only the second series show any diminution of mortality beyond this range. Hence it is concluded that preparations of the nucleo-proteid from solution A may give indications of immunising properties in doses of .01 mg., but the different preparations are not consistent in this respect.

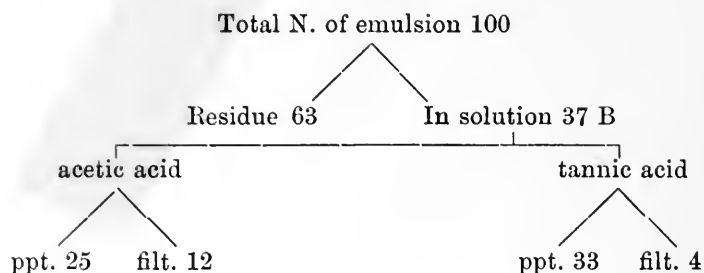
Substance B.

The organisms that have been extracted with water or dilute saline solutions can by appropriate treatment be made to yield a further substance in solution ("*Substance B*"). This treatment, the reason for which may not appear quite obvious, originated, as explained above, in the course of an examination into the various means available for killing

the plague organism without injuring its antigen. Amongst such means dehydration was investigated, and amongst other dehydration methods the use of anhydrous sodium sulphate suggested itself. It was soon found that this reagent was of the greatest utility and that it could be employed with advantage even on cultures already killed. The method found to be the most useful is as follows:—

200 Roux flasks containing neutral agar (1 % Lemco, 2 % peptone, 3 % agar) are inoculated into the condensation water, incubated for 24 hours at 32° C. and the condensation water swept over the surface of the agar by tilting the bottle, by which means a uniform surface inoculation is obtained. Incubation at 32° C. is continued for 4 days. About 1 c.c. of chloroform is then introduced into each flask and the flasks left in the hot room till the next morning. About 10 c.c. of dilute salt solution are squirted into each flask and the surface growth emulsified with a glass rod. The emulsion is drawn by a syphon arrangement into a large flask. This flask contains, therefore, dead bacilli, dilute culture fluid, products of growth and the substance A which we have seen goes into solution on the death of the organisms. The contents of the flask are centrifugalised in a special form of machine (vide Appendix), and the pasty mass of organisms obtained is re-emulsified in salt solution. This emulsion is passed through fine linen to remove chance lumps of agar, and again centrifugalised, the washing in salt solution being repeated if thought necessary. The paste of organisms finally obtained contains about 80 % of water and 20 % solids (dried at 105° C.). This paste is thoroughly mixed in a mortar with finely powdered anhydrous sulphate of soda. Pounding is continued until a dry powder is obtained which is set aside in an ice chest until the next day. The powder is then warmed to 37° C. when it becomes a semi-fluid mass, well stirred, and again set in the ice chest. This alternate cooling and heating is repeated several times. Finally sufficient water is added to dissolve the sulphate of soda, and to make a saturated solution at 37° C. The bacterial bodies are filtered off through hardened paper at 37° C. and suspended in water, whereupon solution of a second part of the bodies takes place. This I call "*Substance B.*"

The nitrogen distribution in this process is as follows. Calling the total nitrogen of the washed emulsion before sulphating 100, 37 parts will be found in solution in the dilute sulphate solution after the process. Of this 37, 25 are precipitated by acetic acid and 33 by tannic acid.

*Chemical Properties of Solution B.*

Solution B is a clear yellowish liquid containing about 5% sulphate of soda in solution. It is faintly alkaline in reaction and holds a nucleo-proteid dissolved in it. When first prepared it is limpid and transparent, but deposits a light cloudy precipitate slowly on standing. On boiling a precipitate is obtained, as also on the addition of acetic acid; the latter precipitate is only thrown down when the solution is distinctly acid. This precipitate is not soluble in reasonable excess of the acid but is very soluble in dilute alkali. The acetic acid precipitate contains organic phosphorus in considerable amount. The filtrate from the acetic acid precipitate also contains some phosphorus. The precipitate contains about 16% of nitrogen.

On dialysis no precipitation occurs. The dialysed solution gives the following reactions. No precipitate is formed on heating, but after the addition of sodium sulphate to the extent of 5% the formation of a considerable precipitate occurs on heating. Acetic acid causes a precipitation of all proteins. 90% alcohol causes no precipitate even on standing, the addition of acetic acid to such a mixture causes an immediate precipitate, which settles in flocculi. The solution gives the biuret (slowly), xanthoproteic, glyoxylic and Mollisch's reaction. It also gives Millon's reaction. The filtrate from a portion that has been precipitated by acetic acid gives no biuret, Millon, glyoxylic or Mollisch's reactions but gives a slight xanthoproteic colouration. From these reactions the conclusion is justified that solution B also contains a nucleo-proteid. This conclusion was confirmed by the recognition of adenin and guanin nitrates as decomposition products. A portion of the precipitate obtained on the addition of acid (acetic) was placed in nitric acid of a density of 1.2 in the ice chest. After a lapse of some days crystals corresponding in appearance to the nitrates of guanin and adenin were recognised microscopically. It has not yet been possible to spare sufficient quantity of the substance to make a more thorough analysis.

Toxic Properties of Solution B.

The following experiments show that when inoculated into rats this substance is weight for weight considerably more toxic than substance A.

Dose mg.	Rat	Result
2·0	{ 1	+ 1 day
	{ 2	+ 1 day
	{ 3	+ 1 day
0·2	{ 4	+ 1 day
	{ 5	+ 1 day
	{ 6	+ 1 day
0·02	{ 7	+ 6 days
	{ 8	+ 7 days
	{ 9	0
0·002	{ 10	+ 9 days
	{ 11	0
	{ 12	0

Lethal dose of the same preparation kept 41 days under toluol at the temperature of the laboratory.

2·0	{ 1	+ 1 day
	{ 2	+ 1 day
0·2	{ 3	+ 5 days
	{ 4	0
0·02	{ 5	+ 2 days
	{ 6	0

Lethal dose of the same sample as the last after heating to 55° C. for 30 minutes.

2·0	{ 1	+ 1 day
	{ 2	+ 2 days
0·2	{ 3	0
	{ 4	0
0·02	{ 5	+ 4 days
	{ 6	0

Lethal dose of the same preparation as the first after keeping for three months under toluol at the temperature of the laboratory.

2·0	{ 1	+ 1 day
	{ 2	+ 1 day
	{ 3	+ 1 day
0·2	{ 4	+ 1 day
	{ 5	+ 1 day
	{ 6	+ 2 days
0·02	{ 7	0
	{ 8	0
	{ 9	0

Lethal dose of another preparation of B.

Dose mg.	Rat	Result
1.0	{ 1	+ 1 day
	{ 2	+ 1 day
	{ 3	+ 1 day
0.1	{ 4	+ 1 day
	{ 5	+ 1 day
	{ 6	+ 1 day
0.01	{ 7	0
	{ 8	0
	{ 9	0

These experiments show that the lethal dose of solution B corresponds to about one-tenth of a milligramme of contained nucleo-proteid; that the solution can be kept under toluol at room temperature for a considerable time without much loss of toxicity; and that heating to 55° C. diminishes toxic power.

The effects observed post-mortem in rats after a fatal dose of this solution are: inflammatory oedema at and about the seat of inoculation and congestion of all the organs of the body, the appearances recalling those observed in rats after death from plague infection but without the buboes.

Effects of Dialysis on Toxicity of Solution B.

Samples of solution B of which 0.1 mg. was a fatal dose were thoroughly dialysed against distilled water. The table below assesses the toxicity of a dialysed solution freshly dialysed, and after 20 and 41 days' keeping under toluol at the temperature of the laboratory.

Dose mg.	Fresh		20 days		41 days	
	Rat	Result	Rat	Result	Rat	Result
7.2	{ 1	+ 1 day	{ 1	+ 1 day	{ 1	+ 1 day
	{ 2	+ 1 day	{ 2	+ 1 day	{ 2	+ 1 day
2.4	{ 3	+ 1 day	{ 3	+ 1 day	{ 3	+ 1 day
	{ 4	+ 1 day	{ 4	0	{ 4	+ 2 days
1.2	{ 5	+ 1 day	{ 5	+ 1 day	{ 5	+ 2 days
	{ 6	+ 1 day	{ 6	0	{ 6	+ 3 days
0.72	{ 7	+ 1 day	{ 7	+ 2 days	{ 7	+ 3 days
	{ 8	0	{ 8	0	{ 8	0
0.24	{ 9	0	{ 9	0	{ 9	0
	{ 10	0	{ 10	0	{ 10	0

Dialysis deprived solution B of some of its toxic power and the toxicity fell still further on keeping. The toxic substance did not pass through the dialysing medium for on evaporating down in vacuo at 15° C. several litres of the dialysate, it was devoid of toxicity.

Toxicity of the acetic acid precipitate from B.

Dose mg.	Rat	Result
1·8	{ 1	+ 1 day
	{ 2	+ 1 day
0·9	{ 3	+ 1 day
	{ 4	+ 3 days
0·18	{ 5	+ 1 day
	{ 6	0

Precipitation by acetic acid thus causes a fall in toxicity. That no toxic substance was left unprecipitated was shown by evaporating a large quantity of the filtrate at 25° C. and failing to find any toxic effect on inoculation into rats. Thus dialysis or precipitation diminishes the toxicity of B. An explanation of this result is not at present obvious.

The effect of salting out solution B.

A solution was prepared containing one lethal dose in one-tenth of a cubic centimetre and sufficient sodium sulphate added to bring the density to 1200. The precipitate was filtered off and dissolved in a volume of water equal to that of the solution before the addition of the salt. The table gives the results of the inoculation into rats of the filtrate and dissolved precipitate.

Dose c.c.	Precipitate		Filtrate	
	Rat	Result	Rat	Result
1·0	{ 1	+ 1 day	{ 1	0
	{ 2	+ 1 day	{ 2	+ 2 days
0·8	{ 3	+ 1 day	{ 3	0
	{ 4	+ 1 day	{ 4	0
0·6	{ 5	+ 1 day	{ 5	+ 2 days
	{ 6	+ 1 day	{ 6	0
0·4	{ 7	+ 1 day	{ 7	0
	{ 8	+ 1 day	{ 8	0
0·2	{ 9	+ 1 day	{ 9	+ 2 days
	{ 10	0	{ 10	0
0·1	{ 11	+ 1 day	{ 11	0
	{ 12	+ 1 day	{ 12	0

Thus sodium sulphate in the strength mentioned (27 grammes of salt to 100 c.c. of water) precipitates the proteins and with them the toxic substance.

This experiment was repeated, the strength of the solution being

19 grammes of salt to 100 c.c. of the solution (density 1150) with these results;

Dose c.c.	Precipitate		Filtrate	
	Rat	Result	Rat	Result
1.0	{ 1	+ 1 day	{ 1	+ 1 day
	{ 2	+ 2 days	{ 2	+ 1 day
0.8	{ 3	+ 1 day	{ 3	+ 1 day
	{ 4	+ 2 days	{ 4	+ 2 days
0.6	{ 5	+ 1 day	{ 5	+ 1 day
	{ 6	+ 2 days	{ 6	+ 1 day
0.4	{ 7	+ 1 day	{ 7	+ 2 days
	{ 8	+ 3 days	{ 8	+ 1 day
0.2	{ 9	+ 5 days	{ 9	0
	{ 10	0	{ 10	0
0.1	{ 11	0	{ 11	0
	{ 12	0	{ 12	0

Sodium sulphate of the strength mentioned distributes the proteins into two portions with an approximately equal distribution of the toxin.

On slowly adding sodium sulphate to solution B of the same protein strength as used in the last two experiments, the first signs of precipitation took place at density 1140 (18 grammes to 100 c.c.). The precipitation limits of the proteid by sodium sulphate are thus density 1140—density 1200.

Filtration of Solution B.

An experiment was made to determine what loss of toxicity occurred on filtration through a close porcelain filter. For this purpose a small Chamberland F. was chosen. Filtration took place rapidly without necessitating much lowering of pressure. The table following gives the toxicity of the solution before and after filtration. No certain loss was observed: the first portions of the filtrate were not rejected. Representing the total nitrogen in the solution before filtration as 100, the total nitrogen in an equal volume of the filtrate was 87, a loss of 13 %.

Toxicity of B before and after filtration.

Dose c.c.	Before		After	
	Rat	Result	Rat	Result
1.0	{ 1	+ 1 day	{ 1	+ 1 day
	{ 2	+ 1 day	{ 2	+ 1 day
0.8	{ 3	+ 1 day	{ 3	+ 1 day
	{ 4	+ 1 day	{ 4	+ 1 day
0.6	{ 5	+ 1 day	{ 5	+ 1 day
	{ 6	+ 1 day	{ 6	+ 1 day
0.4	{ 7	+ 1 day	{ 7	+ 1 day
	{ 8	+ 1 day	{ 8	+ 2 days
0.2	{ 9	+ 1 day	{ 9	+ 1 day
	{ 10	0	{ 10	+ 1 day
0.1	{ 11	+ 1 day	{ 11	0
	{ 12	0	{ 12	0

Immunising value of Solution B.

It has been shown that solution B possesses marked and constant toxic properties and that the toxicity is closely associated with the presence of a nucleo-proteid in solution. It will now be shown that it can be used as a highly efficient vaccine.

The rats in the series of experiments below were each injected with the material in a single dose of the amount mentioned. Fourteen days later their resistance was tested by the subcutaneous inoculation of $\frac{1}{10}$ th c.c. of the broth culture of plague (see p. 542):

Series	Dose mg.	No. of rats	Mortality	% mortality	Minimum % mortality which might be expected once in 43 times (See p. 546 above)
1	0·02	23	2	9	59
2	0·02	29	5	17	61
3	0·01	18	1	6	60
4	0·003	20	4	20	59
5	0·001	15	2	13	55
6	0·0001	24	9	38	59

The effect of heating the solution before use (55° C.) for 30 minutes is brought out by the results of the next series of experiments:

7	0·02	20	6	30	59
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This series should be compared with series 1 which was vaccinated with the same solution but not heated.

Series 2 was vaccinated with a solution that had been kept for three months at the temperature of the laboratory.

The fall in mortality after the test inoculation with plague which occurred in those series of rats which had previously been treated with 0·0001 to 0·02 milligrammes of the protein contained in solution B, is far in excess of any error due to chance (see p. 546 above), and shows that a single injection of even minute quantities had conferred upon them a substantial immunity.

The greater part of the antigen evidently passes into solution after submitting the organisms to the sulphating process. It will be seen later that the residue if sufficiently washed possesses no immunising properties.

The degree of protection obtained with the substance in solution after treatment of the bacilli with anhydrous sulphate of soda compares very favourably with that obtained by the injection of the whole bacillus killed with chloroform and subsequently washed with water. A preliminary series of experiments indicated that 0·03 mg. of such washed

bacilli was the optimum protective dose. The results of two series of rats injected with this dose are given below:

Series	Dose mg.	No. of rats	Mortality	% mortality	Minimum % mortality which might be expected once in 43 times (See p. 546 above)
1	0.03	34	9	27	61
2	0.03	28	7	25	60

The mortality in these series after .03 mg. of bacillus was considerably greater than that obtained with rats protected by .001 mg. of the proteins in solution B.

The Residue of Sulphated Bacilli after Extraction of the Constituents soluble in Saline.

On the completion of the sulphating process, after the final extraction of the sulphated bacteria with water, there remains a residue which consists of the bodies of the bacteria from which those substances contained in solutions A and B have been dissolved out. Examined unstained with the microscope, using dark ground illumination, these extracted bodies do not appear to differ materially from the unextracted organisms. They are however profoundly modified. In the first place, they will take no stain except hot and strong fuchsin; and secondly, their physical characters are changed. Whereas a fresh bacillus examined on a dark ground in water will stand fairly violent treatment and remain intact, a body of a sulphated bacillus is very fragile and slight pressure on the cover glass causes its disintegration. It was at first thought that the effect of the repeated dehydrations of the sulphate process was to rupture the organism and thus allow of the escape of its contents, but this is apparently not the case. Indeed one of the ideas which originated the process was that by means of the strains set up in the alternating fusion and crystallisation the organism might be mechanically ruptured. It would appear however that the treatment modifies the consistence and permeability of the bacterial envelope. Whatever the explanation of the process may be, there is no doubt that a profound alteration in the organism is brought about, and that subsequent to this treatment the proteins dissolved out carry with them a larger proportion of toxic and immunising substances.

The residue consists of material insoluble in water or saline solution. About 17 % is soluble in 1 % Na_2CO_3 and a further quantity in caustic alkali. It contains 10—11 % of nitrogen and some phosphorus in organic

combination, but the amount has not yet been estimated. The residue accounts for 45 % of the total nitrogen of the bacillus. It possesses very small toxicity for rats and the more completely it has been extracted the less poisonous it is. In one case 22 mg. killed one out of two rats, and 7 mg. failed to kill either of two rats. Corresponding with this absence of toxicity there is an absence of immunising power as may be seen by the following experiments :

Series	Dose mg.	No. of rats	Mortality	% mortality	Minimum % mortality which might be expected once in 43 times (See p. 546 above)
8	0·1	39	27	70	62
9	0·03	42	36	86	62
10	0·01	45	38	85	63
11	0·001	44	35	80	63

Even 1000 times the quantity which in the case of solution B afforded a substantial protection, failed to indicate any advantage over the control series.

Another way in which the influence of the sulphating process in facilitating the dissolving out of the toxic substances in the bacilli is strikingly demonstrated is by comparing the toxicity of the residue after

- (1) simple extraction with water,
- (2) sulphating and subsequent extraction.

This is shown in the experiments below, from which it will be seen that the former leaves a highly toxic remainder, whereas the latter exhibited no toxicity within the limits investigated.

Toxicity of an emulsion killed by chloroform, after extraction by water.

Dose mg.	Rat	Result
2·7	{ 1	+ 1 day
	{ 2	+ 2 days
1·4	{ 3	+ 3 days
	{ 4	0
0·7	{ 5	+ 2 days
	{ 6	0
0·3	{ 7	+ 3 days
	{ 8	0
0·1	{ 9	0
	{ 10	0

Toxicity of an emulsion killed by chloroform, after extraction by water, sulphating, and again thoroughly extracting in dilute saline.

22·0	{ 1	+ 2 days
	{ 2	0
7·0	{ 3	0
	{ 4	0
3·0	{ 5	0
	{ 6	0
1·0	{ 7	0
	{ 8	0
0·1	{ 9	0
	{ 10	0

The bulk of the antigen passes into solution only after the treatment of the bacillus with anhydrous sulphate of soda. It is unlikely that this solution contains only the immunising substances, but I may be permitted to point out here the advantages to be anticipated by the use of an antigen in solution :

- (i) Ease of standardization.
- (ii) Greater accuracy of dosage.
- (iii) Sterilization by filtration.
- (iv) Smallness of dose.
- (v) Absence of associated substances of no vaccinating value, which may be undesirable and, at any rate, must be dealt with by the organism before the contained antigen is liberated.

It is now my pleasant duty to thank those who have helped me to attain these results. In the first place I desire to record the loyal assistance of my two laboratory attendants, H. Bray and J. Whittingham; and to express my thanks to my colleagues at the Lister Institute, Dr A. Harden, F.R.S., Dr A. T. MacConkey, Dr A. B. Green and Miss H. Chick, D.Sc., each of whom has at one time or another helped me with advice on technical points. It is difficult to express in a few lines my indebtedness to Dr C. J. Martin, F.R.S., Director of the Institute, whose counsel and practical help have been continually at my disposal throughout the investigation.

CONCLUSIONS.

1. Washing the living organism with chloroform water, while killing the cell, removes a certain amount of a nucleo-protein (substance A) but only traces of the substances which are toxic and immunising for rats.
2. Organisms which have been so treated are toxic for rats and possess immunising power for rats.
3. By appropriate treatment (sulphating process) a further protein substance (substance B) can be dissolved out by water or weak salt solution.
4. This further substance also consists largely of nucleo-protein but contrasts with substance A in respect of its greater and more constant toxicity and of the extent of immunity which it is capable of conferring on rats.

5. Organisms which have had this second substance removed from them are no longer toxic or capable of conferring immunity on rats.

6. There is an intimate association between the toxicity and immunising value of the solution obtained after the sulphating process and both these properties are closely related to the presence of a nucleo-protein, but there is reason to believe they may be found to be independent of it.

APPENDIX.

In order not to interrupt the main argument of this paper by laboratory details they are collected here.

Strain of organisms. For all the experiments, with the exception of testing for immunity, the culture employed was the stock culture of the Lister Institute. It is the culture employed by the Institute in the preparation of Haffkine's prophylactic, and for the preparation of Yersin's serum. It is not very virulent, but is capable of having its virulence raised by passage. Tested for sugar reactions in Durham's tubes it gave

			Acid	Gas
Glucose	+	0
Lactose	0	0
Cane sugar	0	0
Dulcite	0	0
Adonite	0	0
Inulin	0	0
Mannite	+	0
Litmus milk	No change of colour.	

Centrifuge. This was specially designed for washing bacteria and obtaining a paste of organisms from an emulsion. It consists of a steel bowl rotating on its vertical axis at a speed of about 7,000 revolutions per minute. The interior is divided by a vane bisecting the cylinder in a vertical plane passing through the axis of revolution and not reaching quite to the circumference. This vane is removable, and serves to ensure rotation of the fluid at the same rate as the bowl in which it is contained. The bowl is provided with a screw-down lid which makes a water tight joint by means of a rubber ring. Such a bowl deposits on the side after 15 minutes all the organisms in an emulsion in the form of a stiff paste. This paste can easily be removed, after lifting out the vane, by a spatula. As obtained in this way the paste commonly contained from 10—20 per cent. dry matter at 105° C.

Method of inoculation. When working with living cultures of virulent plague and large number of rats, precautions are necessary. A technique was devised which secures maximum immunity against infection both to operator and assistant. An all-glass syringe was fitted on a stand which could be firmly clamped to the table. The nozzle of the syringe was connected by some two feet of small sized pressure tubing with a needle. The piston of the syringe was arranged to be advanced by means of a screw, which was of such a pitch that one turn of the handle expelled one-tenth of a cubic centimetre of a living culture. The rat was held by an assistant, his left hand grasping the neck and forelegs, his right hand holding the right hind leg and the tail. The left hand of the operator holds the left hind leg of the rat. The operator with his right hand pushes the needle through the skin *and muscles* of the left thigh of the rat until the point of the needle impinges under the skin on the other side of the thigh, the skin being raised by the needle but not perforated. One turn of the handle of the syringe now introduces 0.1 c.c. of culture, pure subcutaneously, on the back of the flank. The area around the point of insertion of the needle is now flooded with lysol and the needle withdrawn into the little pool of lysol left, ready for the next rat. The celerity with which all this can be performed is surprising. Generally about three rats per minute received their dose. No regurgitation is possible owing to the muscles interposed in the track of the needle.

Note on Preservatives. During the numerous and lengthy experimental manipulations of the bacillary emulsions or proteid fluids obtained from bacilli it is difficult to altogether avoid contamination with aerial organisms, so that the addition of some preservative is advisable. It was found that the best all round preservative for this purpose was toluol. Thymol was tried, but as it was found to destroy the vaccinating value of an emulsion its use was abandoned. This point is brought out in the following tables which compare the vaccinating value of two emulsions, one preserved with toluol and the other with thymol.

Toluol preserved emulsion.

Series	Dose mg.	No. of rats	No. died	% mortality
I	0.03	34	9	27
II	0.03	28	7	25

Thymol preserved emulsion.

III	0.03	36	24	67
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Coincidentally with this loss of immunizing power there is a considerable fall in toxicity, as the following table shows:

Toluol			Thymol		
Dose mg.	Rat	Result	Dose	Rat	Result
3.0	{ 1	+ 2 days	6.0	{ 1	+ 1 day
	{ 2	+ 2 days		{ 2	0
1.5	{ 3	+ 2 days	4.0	{ 3	+ 4 days
	{ 4	0		{ 4	0
0.75	{ 5	+ 2 days	2.0	{ 5	0
	{ 6	0		{ 6	0
0.3	{ 7	+ 2 days	1.0	{ 7	+ 4 days
	{ 8	0		{ 8	0
0.15	{ 9	0	0.5	{ 9	0
	{ 10	0		{ 10	0

ON THE PRESENCE OF FREE PROTEID IN CULTURES OF PLAGUE BACILLI.

In the light of the knowledge gained in the course of this work, the following observations on the presence of free proteid in broth cultures of the plague bacillus are interesting.

In a young (4 days) culture no coagulable proteid can be found, but after 14 days' growth a considerable quantity was observed, in one case in which the amount was estimated as much as 14 milligrammes per 100 c.c. of culture. In the case of a 13 months' old culture a much larger quantity was found, but the amount was not determined. This proteid material presumably came from the bodies of the bacilli. It should be remembered that in a culture of any organism there are always present a certain proportion of dead individuals and that this proportion increases as time goes on. It would appear then that on the natural death of the bacillus there is an extrusion of some of the contents of the bacterial body. Now it has been shown that there is a close association between the toxic substance contained within the plague bacillus and a nucleo-proteid, and many observers have recorded the fact that the filtrates of old cultures are toxic.

Putting these facts together the conclusion is reached, that the toxic substance obtained with the aid of the extraction methods described in this report is probably identical with that obtained in the filtrates of Dean¹ and Markl².

¹ Dean, *loc. cit.*

² Markl, *loc. cit.*

XXXIX. INTERIM REPORT OF THE ADVISORY COMMITTEE FOR PLAGUE INVESTIGATION IN INDIA.

The following statement embodies the chief conclusions which have been provisionally reached by the Advisory Committee as the result of the investigations made under their direction from 1905—1909 into the mode of spread of plague in India.

(1) Considerable epidemics of human plague consist almost entirely of cases of bubonic plague and are directly dependent on the occurrence of epidemic plague in rats. The development of the rat epidemic precedes the human epidemic by an interval of about a fortnight. There is no evidence that any animals except rats play an important part in plague epidemics.

(2) *Epidemic plague in rats.*

(a) Rat fleas which have sucked the blood of a plague-infected rat can transmit the disease to healthy rats to which they are transferred. The plague bacilli multiply in the stomach of the flea, and the flea may be still capable of conveying infection three weeks after having imbibed plague-infected blood.

(b) If plague-infected rats are kept in close confinement along with healthy rats, no epidemic of the disease occurs in the absence of fleas. In the presence of rat fleas the disease spreads from the infected to the healthy animals, and the rapidity and severity of the epidemic so produced is in proportion to the abundance of fleas.

(c) Rats may be infected by feeding them upon the bodies of other rats dead of plague. The distribution of the lesions in the bodies of naturally infected rats corresponds with that in rats experimentally infected by means of fleas and not with that in rats infected by feeding.

The Committee, therefore, conclude that *in nature plague is spread among rats by the agency of rat fleas.*

(3) *Epidemic plague in man.*

(a) Bubonic plague is not directly infectious from man to man as is shown by the experience of plague hospitals where there is no tendency for the disease to spread from the sick to the attendants.

(b) Material epidemics of plague in man are always associated with epidemic plague in rats. Epidemic plague among rats provides a large number of infected rat fleas, and, owing to the mortality among the rats, brings these fleas on to human beings.

(c) Rat fleas (*Pulex cheopis*) bite human beings, especially in the absence of their natural host.

(d) Rat fleas containing plague bacilli and found capable of transmitting plague to animals may be caught in plague-infected houses.

(e) Animals susceptible to plague (guinea-pigs, monkeys) placed in plague-infected houses if unprotected from fleas may contract the disease; whereas such animals under the same circumstances remain free from plague, if protected from fleas.

(f) The Commission have also performed numerous experiments with a view of testing other possible modes of infection, and have found that:

i. In the absence of fleas no epidemic resulted when animals susceptible to plague (guinea-pigs) were kept in close contact with infected animals although the animals took their food off floors grossly contaminated by the excreta of their infected companions.

ii. Susceptible animals (guinea-pigs) caused to live upon and feed off floors artificially saturated with plague cultures failed to contract the disease.

iii. The excreta of plague-infected patients may contain plague bacilli, but the bedding etc. of plague patients soiled with excreta containing plague bacilli was not found to be infective to highly susceptible animals caused to live in and upon the bedding.

The Committee, therefore, consider that *in the great majority of cases during an epidemic of plague man contracts the disease from plague-infected rats through the agency of plague-infected rat fleas.*

(4) *The Seasonal recurrence and spread of plague.*

(a) The Committee has obtained no evidence that under ordinary conditions the plague bacillus survives for more than a few days outside the bodies of men, animals or fleas.

(b) In large towns plague may persist throughout the year since a few cases of acute plague in men and rats occur during the non-epidemic plague season.

(c) In villages there is no satisfactory evidence that such persistence is of other than exceptional occurrence and it seems probable that the recurring annual epidemics in such places are due in most cases to fresh importation of the infection.

(d) There is no evidence that plague infection is carried for more than short distances by the spontaneous movement of rats. Plague appears to be commonly imported into a fresh locality about the persons of human beings, though the transference of infected rats and fleas in merchandise must be considered.

(e) In districts which suffer annual epidemics of plague, the rat epidemic, on which the human epidemic depends, occurs during some part of that season when the prevalence of fleas is greatest.

TYPHOID FEVER AND MUSSEL POLLUTION.

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1 Chart.

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THIS paper deals with the evidence which has accumulated in Birmingham between June 1st, 1904 and June 1st, 1909, both dates inclusive, as to the relationship between the consumption of mussels and the occurrence of typhoid fever. In Birmingham when a case of typhoid fever is notified careful enquiries are made by trained inspectors as regards, among other matters, the date of onset and the origin of the disease, and the results obtained are verified by the Assistant Medical Officer of Health who visits the house subsequently. These later visits

are of importance as they enable householders to give information which either they had forgotten or were not in possession of at the time of the first visit. By this method of enquiry, which has been instituted by Dr John Robertson, the Medical Officer of Health, it may fairly be claimed that little of etiological importance in connection with the cases of Typhoid Fever which occur in the City escapes attention. During the five years under review 946 cases of typhoid fever, exclusive of cases occurring in public institutions, commenced. Of this number 855 were primary and 91 secondary cases in households.

The etiology of the 855 primary cases in households may be summarised thus:

Cases probably due to personal contact	...	33
Cases probably due to mussels	124
Cases probably due to other shell-fish	...	32
Cases probably due to watercress	5
Cases of unknown origin	661
Total		855

Histories of mussel infection.

In all cases investigated the question of personal contact was first considered. Enquiries were then made as to whether or not mussels or other shell-fish or watercress had been consumed within four weeks of the onset of the illness. In this way a history of mussel eating as the probable source of infection was obtained in 124 out of 855 or 14·5 % of primary cases in households investigated.

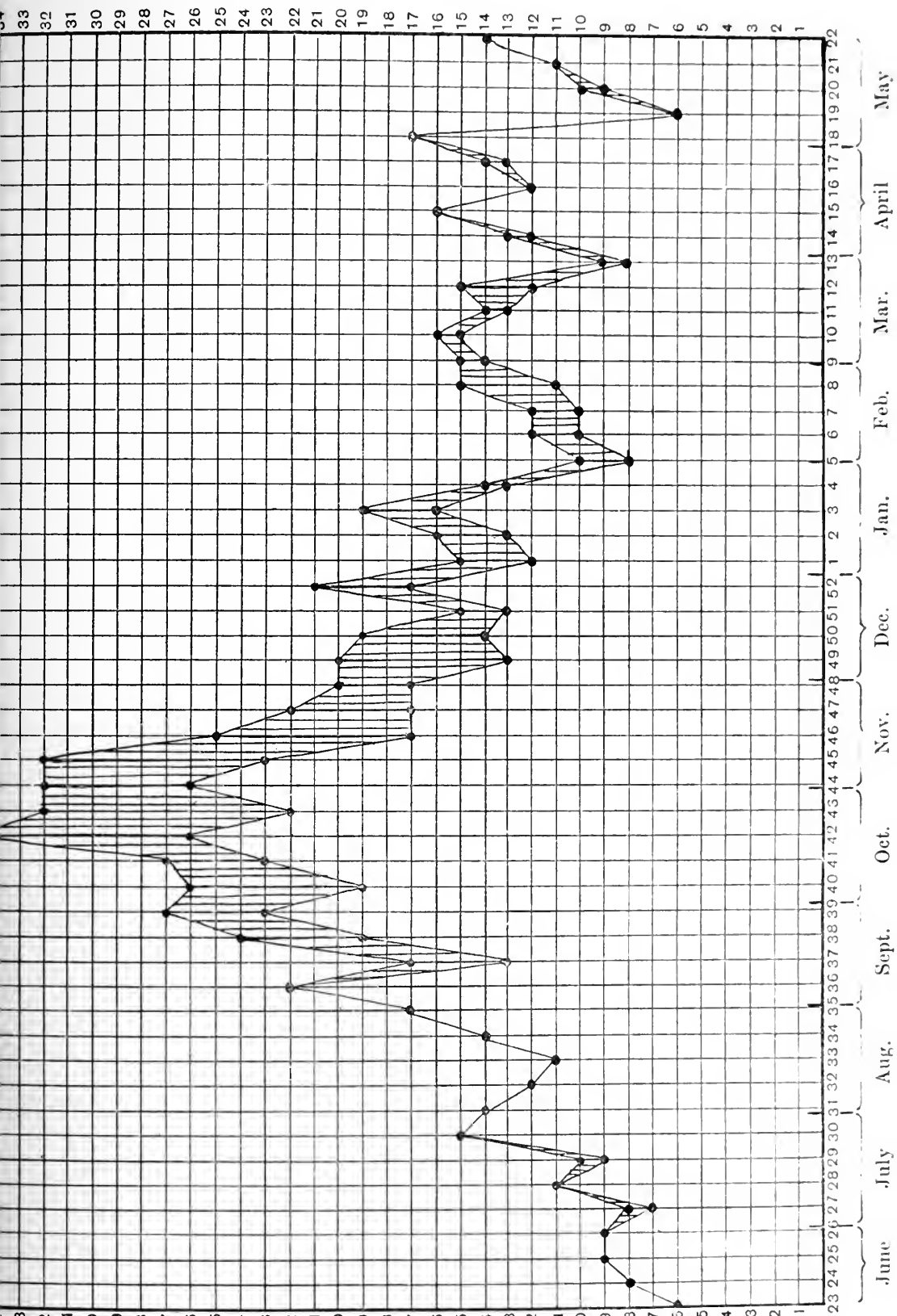
In several cases the histories of the attacks were particularly striking and practically conclusive of mussel infection:

1. It was thrice found that two persons eating mussels from the same batch at the same time fell sick of typhoid fever subsequently within a few days of each other.

2. In six instances persons eating mussels became ill suddenly with vomiting and diarrhœa shortly thereafter, remained unwell, and shewed the signs of typhoid fever a few days later.

3. A boy ate mussels at a watering place. He brought some home to his mother who immediately after eating them was seized with colic and diarrhœa which lasted three days and left her very weak for some time. The boy developed enteric fever.

Chart shewing the 855 primary cases of enteric fever in households in Birmingham commencing between June 1, 1904 and June 1, 1909, both dates inclusive, arranged according to their weeks of onset. The cases of mussels infection are shaded.



4. Upon two occasions it was found that only one person in a family ate mussels and only that person took typhoid fever.

5. In two instances during the close season mussels were gathered from a foreshore known to be polluted and partaken of by holiday makers who fell ill of typhoid fever a few days afterwards.

Curve of mussel-typhoid cases.

Further evidence is adduced in favour of the consumption of polluted mussels as a cause of typhoid fever by considering the curve of enteric fever in Birmingham during the five year period now under consideration. For this purpose the 855 primary cases of typhoid fever in households, whose onsets occurred between June 1st, 1904 and June 1st, 1909, have been plotted out according to their weeks of commencement, the 53rd week in the year 1907 being omitted. By summation of the cases commencing in corresponding weeks the enteric curve of the primary cases in households for the five year period 1904—1909 is obtained as shewn in the accompanying chart. The cases due to mussels have been shaded in and it will be noted that two cases occurred in the 27th and 29th weeks during the close season for mussels. These are the cases above referred to. They may be reckoned as accidental and without bearing on the general features of the chart. The first cases due to mussels have their onset in the 37th week about a fortnight after the beginning of the mussel season on September 1st. Thereafter the number of mussel cases commencing in any one week rises rapidly to a maximum about the 43rd week, remains fairly constant to about the 50th week and thereafter slowly declines. The last case occurs in the 20th week about a fortnight after the mussel season closes. This curve tallies closely with the number of mussels imported into Birmingham. After rapidly rising to a maximum at the commencement of the season the importation of mussels is fairly constant till before Christmas and thereafter gradually falls till the close of the mussel season on April 30th. A glance at the chart will shew that mussel infection certainly accounts for a not inconsiderable portion of the autumnal rise of enteric fever.

Sources of the Birmingham mussel supply.

The value of the statistical evidence which has been detailed associating a certain proportion of cases of typhoid fever occurring in Birmingham with the eating of mussels is enhanced by enquiries which

were made into the sources of the Birmingham mussel supply. Several of them are known to be polluted and in point of fact the sale of mussels gathered from certain layings from which these shell-fish are sent to Birmingham has been prohibited in the London market by the action of the Fishmongers Company.

The contamination of shell-fish layings around our coast has been investigated by Bulstrode (1894—95, pp. 1—108) and Browne (1904, pp. 1—82), and the conclusion that these workers have arrived at on topographical grounds is that there is a number of shell-fish layings which are exposed to pollution gross in amount and also a further considerable number the purity of which is open to doubt.

The probability of sewage becoming at times charged with infective bacilli has been recognised for some time. Recently acquired knowledge as to "bacillus carriers" forces us to conclude that this probability is greater than was originally supposed. About 2—5 per cent. of typhoid convalescents become "chronic carriers" of the infection, discharging the specific organism of the disease in the urine or faeces or both. It would therefore appear that sewage must contain more or less constantly in greater or lesser numbers living typhoid bacilli which may be taken up by mussels placed in proximity to sewer outfalls, and such mussels may obviously produce typhoid fever under favourable conditions in the consumer.

The bacteriological investigation.

In Birmingham it was impossible for various reasons to trace the sources and to obtain for examination samples of the mussels which were held to be responsible for the 124 mussel cases of typhoid fever recorded. It was therefore decided to obtain samples of mussels as they arrived in the Birmingham market and ascertain by bacteriological analyses the amount of pollution, if any, which they contained. This was done from time to time between February 14th, 1908 and March 17th, 1909 and in this way 65 samples of mussels from 22 different known sources were examined by me.

Method of examination of mussels.

Ten fresh living mussels were selected from each sample and opened with a sterile knife after thorough cleansing of the mussel shells in running Birmingham tap water. Care was taken not to lose any of the liquor which was poured into a sterile cylinder of 1000 cubic centimetres

capacity. The bodies were cut into very small pieces over the mouth of the cylinder and allowed to drop in. Note was made of the volume of the shell contents in every case. Sterile water was then added in small quantities at a time with stirring and emulsification and the volume made up to 1000 cubic centimetres. Varying quantities and dilutions of this emulsion were used for the tests employed.

The bacteriological tests employed.

These included the following:

1. An enumeration of the organisms capable of growing on nutrient gelatine (reaction + 1 %) at 20°—22° C. in 3 days.
2. An enumeration of the organisms capable of growing on nutrient agar (reaction + 1 %) at 35°—37° C. in 2 days.
3. An estimation by decimal methods of the number of *glucose-fermenters* present, and an investigation of the nature of these organisms by the use of Bile-Salt Lactose Agar medium and Houston's (1907, pp. 48—52) Quintuple Preferential Method.
4. An estimation by decimal methods of the number of spores of *Bacillus enteritidis sporogenes* present.
5. An estimation by decimal methods of the number of *Streptococci* present and a microscopical examination of these organisms.

The advantage of the method of examination and the bacteriological tests employed in this enquiry may be stated as follows:

1. The volume of the shell contents is recorded for each sample of ten mussels.
2. The entire contents of the shell are submitted to analysis: this is important from the etiological point of view, as a consumer, especially of raw mussels, makes no selection of liquor, body or internal juices.
3. The analysis is quantitative and the results may be expressed per mussel or per cubic centimetre of a mussel.
4. The analysis is also qualitative as regards certain organisms known to be indicative of sewage pollution.

Mussel bulk.

The total volume of the 650 mussels examined was 8750 cubic centimetres equal to 13.46 cubic centimetres per mussel. The smallest volume of a sample of 10 mussels noted was 53 c.c. and the largest

250 c.c., and it may be said at once that no relationship could be traced between the size of the mussels, their bacteriological content and the period of the season at which they were gathered.

The number of organisms in the samples examined.

For these counts it was necessary to dilute the emulsion of mussels and sterile water prepared as stated. One c.c. of the emulsion was put into 9 c.c. of distilled water and thoroughly shaken and 1 c.c. of this mixture was diluted and re-diluted in a similar manner six times. The 4th and 6th dilutions, in which 1 c.c. equalled respectively $\cdot 000001$ and $\cdot 00000001$ of a mussel, were examined in every case for these enumerations.

Gelatine count. This count varied greatly for the different samples examined. The smallest count obtained was 2,000,000—upon two occasions—and the highest more than 13,700,000,000 bacteria per mussel and recorded as “innumerable”—also upon two occasions. Excluding these unnumbered counts the average number of bacteria per mussel for the remaining 63 samples was found to be 1,172,000,000. This average is accounted for mainly by the samples yielding more than 1,000,000,000 colonies per mussel, viz. 14, excluding which gives an average count for the remaining 51 samples of 139,000,000 bacteria per mussel.

Agar count. This was nearly always less than the corresponding gelatine count. The smallest count with this test was 2,000,000 obtained upon four occasions and the highest more than 5,000,000,000 and called “innumerable.” Excluding this count we find that the average agar count for the remaining 64 samples is 411,000,000 bacteria per mussel. In eight instances a count of 1,000,000,000 or more was recorded. If these eight samples are excluded the average count for the 57 remaining samples is 86,000,000. The following table classifies the results of the counts.

	Gelatine count	Agar count
No. of samples yielding between 1 million and 10 million organisms per mussel	12	14
No. of samples yielding between 10 million and 100 million organisms per mussel	20	30
No. of samples yielding between 100 million and 1000 million organisms per mussel	19	13
No. of samples yielding over 1000 million organisms per mussel	<u>14</u>	8
Total	65	65

The above classification shews that all the samples of mussels examined as they arrived in the Birmingham market contained a large number of micro-organisms. The most likely number for any sample of mussels to contain was between 10 million and 100 million per mussel and this must be considered high. It is certain that samples shewing a larger number than this are polluted and in those cases where the number is over a total of 1000 million per mussel gross pollution undoubtedly exists. It is indeed a question if all the samples examined were not dangerous from the point of view of the public health, but this is a matter which cannot be definitely settled until we possess full information as to the bacterial content of mussels cultivated in regions remote from all possibility of contamination.

The nature of the organisms in the samples examined.

The next part of the investigation was concerned with the estimation of the numbers of certain organisms known to be indicative of sewage pollution.

Glucose-fermenters. After definite quantities of the mussel emulsion had incubated in Glucose Bile-Salt Broth at 35°—37° C. for 48 hours plates were made on Bile-Salt Lactose Agar from those tubes shewing the formation of acid and gas with the smallest quantity of mussel emulsion. The characters of the colonies growing on Bile-Salt Lactose Agar medium were noted and five colonies, red in colour if possible, were selected from each plate and their reactions tested by Houston's Quintuple Preferential Method for fermentation of glucose, lactose and saccharose and production of indol and fluorescence. For the simpler classification of these results a numerical value was assigned to each test in accordance with the following scheme :

Fermentation of glucose	= 2
Fermentation of lactose	= 1
Production of indol	= $\frac{1}{2}$
Production of fluorescence	= $\frac{1}{4}$
No change in saccharose	= $\frac{1}{8}$

This method has the advantage that by its means the results are easy to classify and at the same time the value assigned indicates clearly the reactions of the organisms, as well as the importance of the organism as regards pollution. Thus *Bacillus coli communis* would have the highest possible value $3\frac{7}{8}$ and the more nearly an organism resembled

this bacillus the more nearly would its value approximate $3\frac{7}{8}$ and the stronger would be the evidence derived from its presence in favour of recent pollution by matter of excremental origin.

The following is a classification of the results on this basis :

No. of glucose-fermenters per mussel			Numerical value assigned to organism isolated	No. of samples	No. of glucose-fermenters per mussel			Numerical value assigned to organism isolated
Between	10,000 and	100,000			Between	100 and	1,000	
„	1,000	„ 10,000	$3\frac{7}{8}$	1	„	10	100	$3\frac{1}{4}$
„	100	„ 1,000	$3\frac{7}{8}$	1	„	1	10	$3\frac{1}{4}$
„	1	„ 10	$3\frac{7}{8}$	1	„	1,000,000	10,000,000	3
„	10,000	„ 100,000	$3\frac{3}{4}$	1	„	10,000	100,000	3
„	1,000	„ 10,000	$3\frac{3}{4}$	1	„	1,000	10,000	3
„	100	„ 1,000	$3\frac{3}{4}$	1	„	100	1,000	3
„	10	„ 100	$3\frac{3}{4}$	3	„	10	100	3
„	1,000	„ 10,000	$3\frac{1}{2}$	1	„	10	100	$2\frac{7}{8}$
„	100	„ 1,000	$3\frac{1}{2}$	1	„	100,000	1,000,000	$2\frac{1}{2}$
„	10	„ 100	$3\frac{1}{2}$	1	„	100	1,000	$2\frac{3}{4}$
„	1,000,000	„ 10,000,000	$3\frac{3}{8}$	1	„	1,000,000	10,000,000	$2\frac{1}{4}$
„	100,000,000	„ 1,000,000,000	$3\frac{1}{4}$	1	„	10,000	100,000	$2\frac{1}{4}$
„	1,000,000	„ 10,000,000	$3\frac{1}{4}$	2	„	1,000	10,000	$2\frac{1}{4}$
„	100,000	„ 1,000,000	$3\frac{1}{4}$	2	„	10	100	$2\frac{1}{4}$
„	10,000	„ 100,000	$3\frac{1}{4}$	1	„	1,000	10,000	2
„	1,000	„ 10,000	$3\frac{1}{4}$					

* In some cases more than one dilution was examined, but for the purposes of this classification only that giving the organism of highest value is taken into account.

From the foregoing classification it is seen that 26 out of 65 samples examined contained *glucose-fermenters* of higher value than $3\frac{1}{2}$. As these organisms indicate recent sewage pollution it is clear that their presence in a sample of mussels must be regarded with grave suspicion from the public health standpoint. In 22 of these 26 samples there were more than 100 of these organisms per mussel, in 13 the number exceeded 1000 per mussel, and in six the number per mussel was over 10,000. In five of the six samples with more than 10,000 *glucose-fermenters* of higher value than $3\frac{1}{2}$ per mussel the organism isolated was actually *B. coli communis*, and bearing in mind the close relationship between this organism and *B. typhosus* as regards habitat it would seem a fair conclusion that specific pollution of mussels with the latter microbe must occasionally occur.

In considering the degree of pollution of the remaining samples it is important to note that not only the value of the organism isolated but

also the number present must be taken into account. It is impossible to say what increase in number makes up for a diminished value in the isolated organism according to the present classification. For the matter of the finer issues it is necessary that this should be defined and as a sequel the "permissible degree of biological impurity" referred to by Houston (1904, p. 170). But even without this knowledge a scrutiny of the classification submitted leaves no room for doubt as to the objectionable nature of the larger number of the samples examined.

It has been urged by Houston (1904, p. 307) that shell-fish (oysters) placed on the market for sale should not be hastily condemned for the following reasons :

- (1) Coli-like microbes may multiply in the oyster,
- (2) Coli-like microbes may diminish in number in the oyster, and
- (3) The oysters may have become contaminated after removal from the fishery.

Johnstone (1909, p. 438) expresses the same opinion for reasons (1) and (3) as above numbered. It would of course be unwise and indeed impossible to condemn a mussel laying on the results of the bacteriological examination of shop samples but such an examination clearly reveals the danger to which the consumer is exposed. The sixty-five samples examined in the present enquiry were obtained upon their entry to the Birmingham market and the large proportion which the test under discussion proves to be grossly polluted indicates the need for restrictions as to layings and subsequent storage before transit.

Bacillus enteritidis sporogenes. The spores of this organism were sought for in every case with the following results :

				No. of spores of <i>B. enteritidis</i> <i>sporogenes</i> per mussel		
5 samples shewed between 1,000 and 10,000						
24	"	"	"	100	"	1,000
31	"	"	"	10	"	100
4	"	"	"	1	"	10
1 sample	"			none		

In dealing with this organism it is to be remembered that it occurs in sewage along with *B. coli communis* usually in the proportion of 1 of the former to 100 to 1000 of the latter, and this proportion should always be borne in mind in estimating the nature of the pollution of any batch of mussels. As we have already indicated that mussels with 1000 *glucose-fermenters* of high value or organisms of the colon group

are dangerous it is clear that mussels with more than 10 spores of *B. enteritidis sporogenes* must be looked upon with grave suspicion. Only five out of the 65 samples contain less than this number of spores per mussel, and this fact is further evidence of the gross sewage contamination of the mussels examined.

Streptococci. The investigation as regards this class of organism has been already outlined. Frequent endeavours were made to isolate *Streptococci* seen microscopically but only on three occasions were the attempts successful. The results of the microscopic examination are as follows :

No. of <i>Streptococci</i> per mussel					
2 samples shewed between 100,000,000 and 1,000,000,000					
2	„	„	10,000,000	„	100,000,000
5	„	„	1,000,000	„	10,000,000
2	„	„	100,000	„	1,000,000
10	„	„	10,000	„	100,000
8	„	„	1,000	„	10,000
7	„	„	100	„	1,000
4	„	„	10	„	100
25	„	„	less than 10		

The above table shews that *Streptococci* were not found in as much as 1 mussel in 25 instances although on other grounds 14 of these samples were bad. These facts combined with our scant knowledge of the nature and importance of *Streptococci* make it impossible to lay down any definite guide with regard to them. But it is clearly established that mussels may contain these organisms in large numbers and so far as they indicate contamination a large proportion of the 65 samples of mussels examined must be considered grossly polluted.

Conclusions based on the bacteriological results.

All the five tests which have been applied have been carried out so that the results could be expressed quantitatively. It is interesting to note that these tests corroborate each other and shew clearly that mussels as they arrive in the market are grossly polluted by sewage organisms. The risk to the consumer has been shewn and as it is impossible in the practical work of administration to use Sections 116—119 of the Public Health Act, 1875 to protect him, the need for further legislation to prevent mussels from polluted sources being sent into the market becomes apparent.

Cooking of mussels.

Various experiments have been carried out in connection with the cooking of shell-fish. Clark and Gage (1905, pp. 427—457) have pointed out that *B. coli* was found in as large a proportion of clams after 15 minutes steaming as in uncooked clams, and that *Streptococci* were found after 30 minutes steaming. The same observers conclude: "The cooking experiments with both clams and oysters shew that some of the common methods of cooking cannot be depended upon to destroy the bacteria of the sewage type found in shell-fish; that is, to do this, a degree of heat or a period of cooking is required in many instances that destroys or impairs the palatability of both oysters and clams. It is, of course, evident that neither should be eaten in the raw condition except from unpolluted and certified sources."

I have been able to confirm these views and at the same time to shew how difficult it is to sterilise mussels.

In the following experiments the mussels used were fresh and the shells were cleansed with a sterile nail-brush in running Birmingham tap water immediately before each experiment was begun.

(1) *Boiling in a saucepan.*

Mussels cleansed as above described were put into a saucepan with Birmingham tap water which was quickly heated to the boiling point. A mussel separated from the shell was taken out immediately the water boiled and put with sterile precautions into a large sterile broth tube and incubated at 35° to 37° C. Boiling was allowed to continue and the process of removing and incubating a mussel was repeated at regular intervals. Growth occurred in all incubated tubes. The following table gives the times of boiling and incubation before growth appeared in nine experiments conducted in this way:

No. of minutes boiling before incubation							No. of hours incubated at 35°—37° C. before growth appeared						
Exp. 1	Exp. 2	Exps. 3 & 4	Exp. 5	Exp. 6	Exps. 7 & 8*	Exp. 9†	Exp. 1	Exp. 2	Exps. 3 & 4	Exp. 5	Exp. 6	Exps. 7 & 8*	Exp. 9†
0	0	8	16	30	40	60	24	24	48	48	48	48	72
4	4	16	26	45	60	80	24	24	48	48	72	48	72
8	6	24	36	60	80	100	48	48	48	48	72	72	96
12	8	32	46	75	100	140	48	48	48	72	72	72	96
16	10	40	60	90	120		48	48	48	72	72	72	
				105							72		
				120							72		

* Exp. 8. After 120 minutes boiling a mussel shell was incubated and proved sterile.

† Exp. 9. After 140 minutes boiling a mussel shell was incubated and proved sterile.

(2) *Heating in steam steriliser at 100° C.*

Each of five cleansed mussels with shells were put into sterile broth tubes and steamed in a steam steriliser at 100° C. The tubes containing the mussels and shells were taken out at intervals, cooled and allowed to incubate at 35°—37° C. A growth always resulted. A broth tube was put on in each experiment as a control and remained sterile after heating and incubating. The following is a statement of the times of heating and incubation before growth appeared in five experiments conducted in this way:

No. of minutes in steam steriliser at 100° C. before incubation			No. of hours incubated at 35°—37° C. before growth appeared		
Exps. 10 & 11	Exps. 12 & 14	Exp. 13	Exps. 10 & 11	Exps. 12 & 14	Exp. 13
30	60	120	48	48	72
40	90	180	48	72	72
60	120	210	48	72	72
80	150	240	72	72	72
100	180	270	72	72	72

All these experiments shew that mussels are not sterilised after heating for considerable periods. After heating for a short time—from 6 to 8 minutes—growth appears in nutrient broth after 48 hours incubation and no better results as regards sterility are obtained by heating for a much longer period. This may mean that heating for these periods kills surface organisms but not deep-seated organisms or spores, or kills surface and deep-seated organisms but not spores especially if these are deep-seated.

In the event of *B. typhosus* being present in the intestinal tract or other deep-seated region of a mussel it may be fairly concluded from the foregoing experiments that the usual method of cooking by no means dispels the risk of infection to the consumer. Klein (1905, p. 46) has arrived at the same conclusion from another standpoint, and Thresh (1906, p. 253) has been able to trace cases of typhoid fever to cockles steamed under pressure for three to five minutes.

Remedies suggested.

The foregoing evidence from the statistical and experimental aspects clearly associates typhoid fever with polluted mussels. Various remedies have been suggested:

(1) Purification of sewage before its discharge into tidal waters. This has been considered by the Royal Commission on Sewage Disposal

(1904, p. xx) and shewn to be impracticable as purified effluents contain large numbers of organisms of intestinal derivation.

(2) Seizure and destruction of unwholesome shell-fish under Secs. 116—119 of the Public Health Act, 1875. The provisions contained in these sections are from an administrative standpoint useless for preventing the sale of contaminated shell-fish as there is nothing in the appearance to distinguish the polluted from the clean.

(3) Removal of the source of pollution. This is a possible method under certain circumstances.

(4) Cleansing polluted shell-fish. The following varying results of observers in this field hold out little hope that this method can be utilised.

(a) Foote (1894, pp. 197—199) recovered *Bacillus typhosus* from experimentally infected oysters kept in a cool room 30 days from the date of infection.

(b) Klein (1894—95, pp. 116—120) was able to recover *B. typhosus* from experimentally infected oysters kept in the laboratory in clean sea water changed daily 17 days after infection.

(c) Chantemesse (1896) added typhoid bacilli to sea water in which oysters were placed for 24 hours. He recovered the organism from the body of the oyster 24 hours after its removal from the water. He recommends a period of several weeks for cleansing.

(d) Herdman and Boyce (1899, p. 46) state that when experimentally infected oysters were subjected to a running stream of clean sea water there was a great diminution or total disappearance of *B. typhosus* in from one to seven days.

(e) Mosny (1899—1900, p. 1098) is of opinion that a period of eight days in pure sea water is sufficient for the disappearance of typhoid organisms from oysters.

(f) Hewlett (1903, p. 166) shewed that typhoid bacilli remain and multiply in cockles placed in sea water.

(g) Houston (1904, p. 299) re-laid polluted oysters in sea water remote from sewage pollution and found *B. coli* still present in considerable numbers after 26 days.

(h) Klein (1905) shewed that oysters infected with huge numbers of *B. typhosus* kept in clean sea water frequently changed were able to clean themselves in six days: that oysters infected with human faecal matter and thereafter kept in sterile sea water frequently changed were able to rid themselves of *B. coli* in eight days; that cockles infected with typhoid organisms and thereafter kept in clean sea water

frequently changed allowed the bacilli to multiply; that in mussels similarly treated the bacilli were still plentiful after seven days.

(i) Johnstone (1909, pp. 436—438) found that mussels taken from polluted beds and placed in sea water half-a-mile from the nearest outfall sewer were able to rid themselves of 93 per cent. of intestinal bacteria in four days, and that a further period of eight days did little if anything to effect a further reduction.

(5) Sterilisation of shell-fish. The results obtained by the experiments conducted during the present enquiry and those of Clark and Gage already quoted shew that this is not reasonably possible.

(6) Control of waters and pits, ponds, layings and beds. This was the remedy suggested by the Royal Commission on Sewage Disposal (1904, p. xxi) and it seems to me the only practical method as none of the other remedies which have been mentioned can be relied upon with certainty to protect the consumer from the risk of infection. Legislation on these lines would have the added advantage that it would ensure the stability of trade interests by the practical guarantee involved that mussels exposed for human consumption were obtained from clean sources.

My thanks are due to Dr John Robertson, Medical Officer of Health for the City of Birmingham, for permission to publish this paper and to Professor R. F. C. Leith, University of Birmingham, in whose laboratory the bacteriological work was carried out.

SUMMARY.

(1) 855 primary cases of typhoid fever in households in Birmingham were investigated.

(2) In 124 or 14·5 % of these cases a history of mussel eating within four weeks of the onset of the disease was obtained.

(3) In 17 instances the histories were conclusive of mussel infection.

(4) The curve of mussel-typhoid cases arranged in their weeks of commencement tallies closely with the importation of mussels into Birmingham.

(5) Mussel infection is one of the causes contributing to the autumnal rise of enteric fever.

(6) Some of the sources from which mussels are sent to the Birmingham market are known to be exposed to dangerous sewage contamination.

(7) 65 samples of mussels obtained on entering the Birmingham market from 22 different known sources were analysed bacteriologically.

(8) The results in terms of (a) the total number of organisms, (b) the number of *glucose-fermenters*, (c) the number of spores of *Bacillus enteritidis sporogenes*, and (d) the number of *Streptococci* shew that pollution to a dangerous degree of a large proportion of mussels placed on the market for human consumption exists.

(9) 14 experiments were carried out in relation to mussels subjected to moist heat at 100° C.

(10) These shew that the ordinary method of cooking mussels does not remove the risk of typhoid infection and that mussels may be heated in a steam steriliser at 100° C. continuously for as long as four and a half hours without sterilisation.

(11) Legislation is necessary to prohibit the gathering of mussels for human consumption from mussel beds exposed to sewage contamination.

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THE ACTION OF RUBBER ON MERCURIAL ANTISEPTIC SOLUTIONS.

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(From the Wellcome Physiological Research Laboratories.)

UNEXPECTED contamination of several jars of a culture-medium was traced to some red rubber stoppers which had been washed and boiled and then kept for some days in 1:1000 potassium mercuric iodide solution, which was exposed to aerial contamination. An examination of this solution showed that it had very greatly diminished antiseptic properties. Not only did a mixture of equal volumes of the solution and broth support the growth of *Staphylococcus* but living organisms were actually present in it. These observations were repeated with a second solution of pure mercuric iodide and potassium iodide and other samples of red rubber, and their confirmation led to the investigation of the action of several kinds of rubber upon potassium mercuric iodide solutions.

The absorption of mercury from 1:1000 biniodide solution was examined quantitatively in the case of the following four samples. For their description we are indebted to the manufacturer.

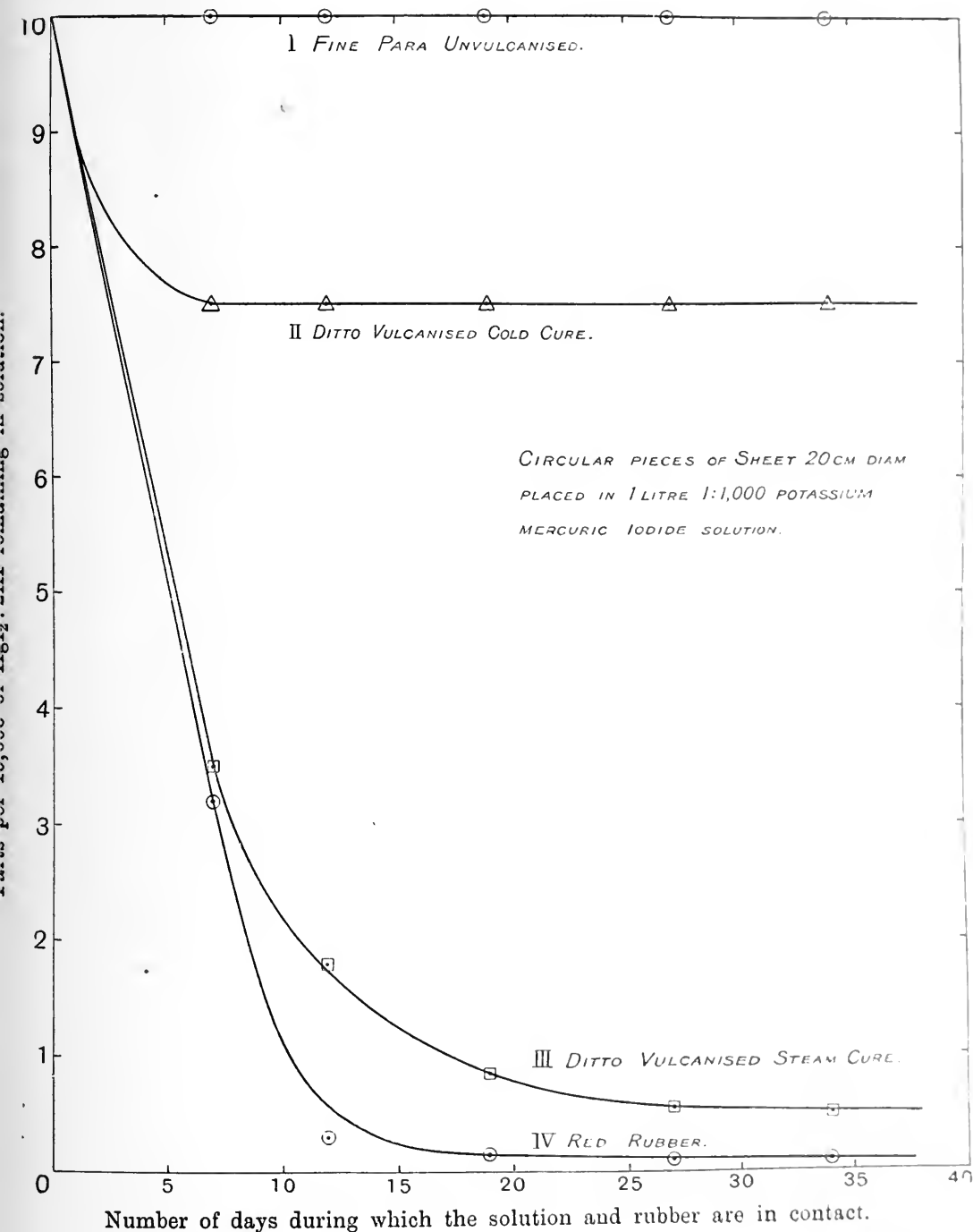
Sample 1. Fine hard Para rubber unvulcanised. In order to get it into sheet form it was necessary to soften it by coal-tar naphtha. It is pure rubber and contains no filling of any kind, and as it is not vulcanised it retains its solubility in chloroform, benzene, etc. It is met with as a manufactured article when rubber is required with its adhesive properties unimpaired.

Sample 2. This is exactly the same as the above, except that it has been vulcanised by the "cold-cure process," i.e. it has been treated with carbon disulphide and sulphur chloride. The best quality black rubber surgical goods, surgical gloves, catheters, etc., are made of this material.

Sample 3. This is the same as 1, except that it has been vulcanised by "steam-cure," i.e. sulphur has been incorporated and the whole heated to 280° F.

Sample 4. This is the ordinary red rubber from which rubber stoppers and tubing are made. It resembles 3 but contains antimony sulphide and sulphur. Some good quality red rubber surgical goods contain vermilion and are cold-cured.

Experimental. The samples were circular pieces of sheeting 20 cms. in diameter. Each piece was superficially cleansed by scrubbing with sand and soap, rinsed in distilled water and transferred to a stoppered bottle containing 0.55 gm. mercuric iodide and 0.45 gm. potassium iodide dissolved in 1 litre of water. From time to time samples of 25 c.c.s. of the contained liquid were removed from each of the four bottles and at the end of 34 days the mercury content of each of the samples was determined. The results are expressed in the following diagram.



The method employed for the estimation of the small quantities of mercury in these solutions was analogous to that used by Harcourt¹ for the estimation of small quantities of lead. 10 c.cs., and, in the case of great dilution, 20 or even 40 c.cs. of the solution to be examined were diluted to 50 c.cs. in Nessler tubes and two drops of a 10% caustic potash solution saturated with sulphuretted hydrogen solution added. The brown tint of the colloidal mercuric sulphide was found to become permanent after ten minutes, and under these conditions there was no tendency to precipitation. Comparison was made with tubes similarly prepared containing known volumes of 1:1000 biniodide solution. Accurate results are obtained, and much time is saved in manipulation, if the standard tubes and the experimental tubes are prepared, and then the sulphide added to all as nearly simultaneously as possible.

It would seem from the results obtained that samples of rubber containing a considerable quantity of sulphur and sulphides take up more mercury than those containing little, while pure rubber takes up no mercury at all. In cases 3 and 4 the rubber surface showed signs of "perishing." It had been observed previously that the walls of a piece of rubber tubing through which 1:1000 potassium mercuric iodide solution had been passed from time to time for some weeks showed a corrosion to a depth of 1—2 mm.

A single experiment performed with 1:1000 mercuric chloride solution and red rubber showed that the mercury was taken up just as it was from a biniodide solution.

Conclusions.

The practical bearing of these results on the sterilisation and sterile storing of rubber catheters, tubing, stoppers, sheeting, etc., by means of solutions of mercuric salts, is important. Not only do the solutions in which manufactured rubber articles are placed for some time lose mercuric salt but the rubber surface is attacked.

Sterilisation of clean rubber articles may be effected by soaking in mercurial solutions, but prolonged immersion leads to exhaustion of the mercury and it becomes imperative to guard against subsequent contamination.

¹ Harcourt (1910), *Journ. of Chem. Soc.*, p. 840.

PLAGUE AMONG GROUND SQUIRRELS IN AMERICA.

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(Plates XII and XIII. 1 Map.)

THE United States has been fortunate in never having had any extensive epidemics of plague. With the exception of a few cases, not over a dozen, that are directly chargeable to the infection of the indigenous rodents (ground squirrels), the disease has been confined to the two largest and most important cities on the Pacific Coast, San Francisco and Seattle. In each city the disease has yielded promptly to vigorous sanitary measures carried out by the public health arm of the Federal Government. Under the political organization of the Government, direct control of measures for the suppression of a disease is taken by the central sanitary authority only when a request is made by the local authorities, but it has been the experience that local authorities are prompt to make requests for assistance whenever any serious epidemic appears.

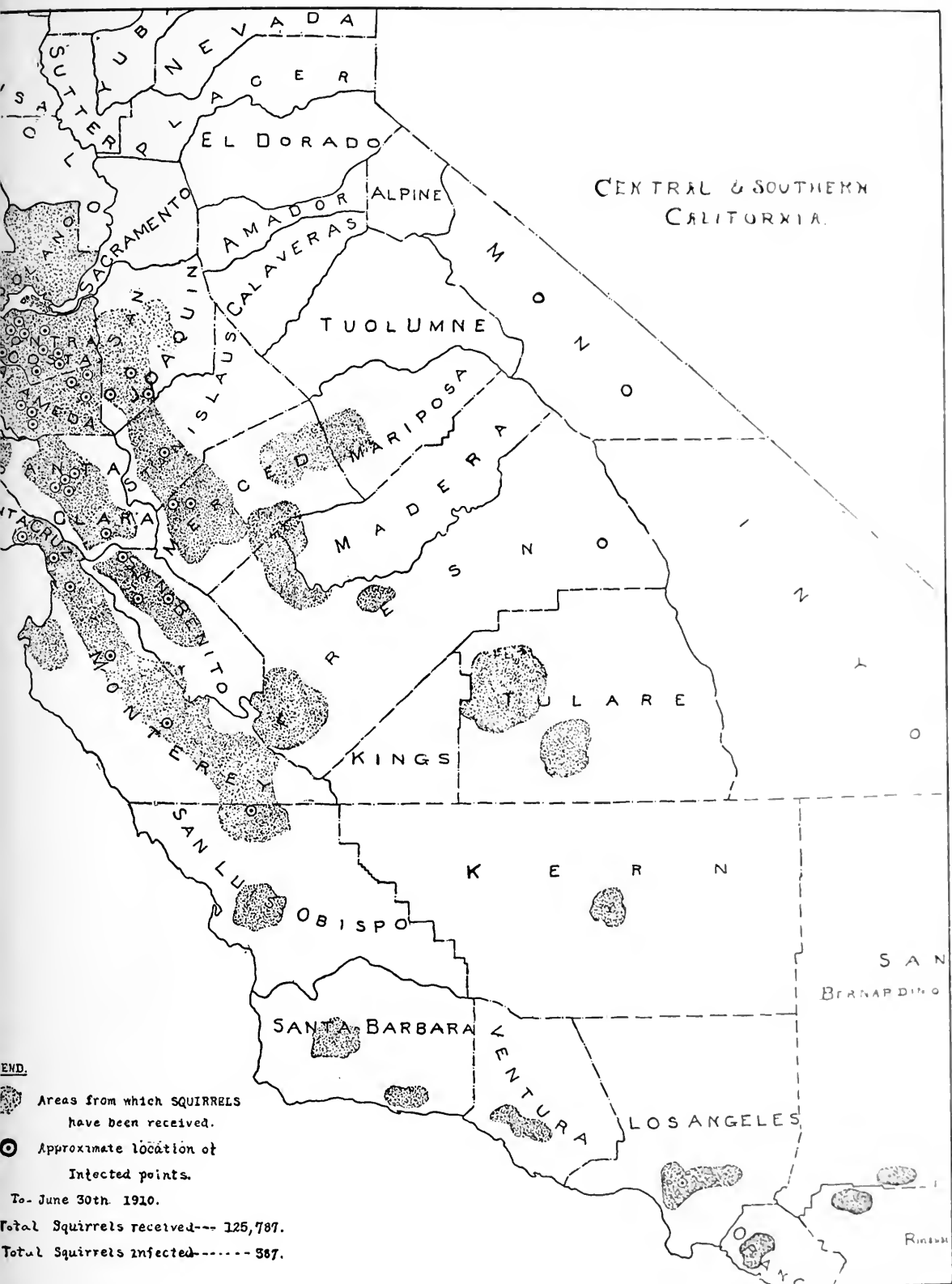
California, it will be recalled, is the State that forms the greater part of the Western Sea Coast of the United States. It lies between $32^{\circ} 32'$ and 42° North Latitude, and $114^{\circ} 30'$ and $124^{\circ} 22'$ West Longitude. Its area is over 156,000 square miles. In size, the State is next to the largest in the Union, and perhaps excels all others in natural advantages, mineral and agricultural wealth, beauty and salubrity. San Francisco, the most important of the large cities, has a harbour equalled by few anywhere and excelled by none, and stands as a natural gateway to the Orient and to all Pacific ports. This port

enjoys an enormous commerce with all parts of the globe. With plague prevailing at a number of Oriental ports, it is scarcely a matter of surprise that even with an efficient maritime quarantine, the infection should have slipped in. There is no evidence that points to any particular vessel as the carrier by which the disease was imported, but it may be safely assumed that infected rats found their way from some vessel to the shore, or less likely, that infected foodstuffs imparted the disease directly to the shore rats.

Plague first appeared in the United States at San Francisco in 1900. There was no direct evidence that the rats were infected until several years later, but in view of what we now know of the relation of rat plague to human plague, we may assume that rodent plague was probably present at least as early as the first human cases. According to Surgeon Blue, U.S.P.H. and M.H.S.⁽¹⁾, the first suspicion of plague among the ground squirrels was aroused in 1903, when an epizootic

Table showing extent of investigation and cases of human plague.

County	Squirrels examined	Infected with plague	Ratio of infection	Human cases of plague of squirrel origin
Contra Costa	31857	245	1 : 130	6
Alameda	8985	85	1 : 105	3
San Mateo	2819	0	—	0
Santa Cruz	1590	3	1 : 530	0
Santa Clara	6813	23	1 : 296	1
San Benito	6239	24	1 : 259	1
Monterey	18855	4	1 : 4713	0
San Luis Obispo	10662	1	1 : 10662	0
San Joaquin	6884	9	1 : 764	0
Stanislaus	1976	5	1 : 395	0
Merced	10408	2	1 : 5204	0
Mariposa	687	0	—	0
Madera	785	0	—	0
Fresno	12726	0	—	0
Tulare	6367	0	—	0
Kern	326	0	—	0
Santa Barbara	4651	0	—	0
Ventura	2189	0	—	0
Los Angeles	10854	1 (1908)	1 : 10854	1
Orange	1792	0	—	0
San Bernardino	577	0	—	0
Riverside	1268	0	—	0
Solano	1070	0	—	0
Napa	114	0	—	0
Colusa	110	0	—	0
Total	150604	402	1 : 374	12



Map showing areas investigated and infected localities, prepared by Assistant Surgeon French Simpson, U.S.P.H. and M.H.S., by whose permission it is published. (San Francisco on the peninsula just north of the county marked San Mateo. Oakland is in Alameda County, just across the Bay, east of San Francisco. Los Angeles is in the centre of the dotted area in Los Angeles County.)

affected so many of these rodents in Contra Costa County, California, that they were almost exterminated. Since that time there has evidently been a great increase among the rodents throughout this county. At about the same time, probably later (the history is not definite), there was a heavy mortality among the ground squirrels in Alameda County, which lies immediately south of Contra Costa County. There is no proof that the epizootic was plague, but as several cases of plague in man occurred in the territory invaded at about the same time, it seems not unlikely that this disease was present among the rodents. The victims were persons who had not been exposed to the possibility of being infected from a known epizootic among rats. This, coupled with the fact that several of these persons were known to have handled squirrels a few days before the onset of the symptoms, led to the suspicion that plague was prevalent among the rodents.

In the summer of 1908, two cases of plague (both fatal) occurred in the squirrel country. A short time after the occurrence of these cases, a dead squirrel was found near the house where one of the victims had lived. This squirrel was submitted to a bacteriological examination by Acting Assistant Surgeon William B. Wherry, U.S.P.H. and M.H.S.⁽¹⁾, and, as the result of his investigations, it was shown to be infected with plague. The case was studied independently by the writer⁽²⁾ with like result. Later in the same year, three other plague infected squirrels were found within a radius of a few miles of the first one.

The Ground Squirrel.

The California ground squirrel, *Citellus beecheyi*, is a burrowing animal that usually lives in colonies composed of from three or four to perhaps thirty members or more. The favourite locality for the burrows of these rodents is in the foothills or rolling country. They generally avoid land that is flooded from time to time. They have been seen by the writer on mountains to an altitude of over 3000 feet, and there are reliable statements of their presence at even higher points. Their food is grain, fruit, nuts, green grass, leaves, and seeds of various sorts. They are very destructive to crops, and consequently farmers and fruit growers wage a continual warfare against them.

The rodents are very prolific. We have counted 133 foetuses in fifteen consecutive females. The young are born in March, April, and May. During the rutting season the sexual glands of the male grow

almost as large as the end joint of the thumb, while during the remainder of the year these organs are scarcely larger than a pea.

The average weight of the adult rodents is about 750 grams; one of the largest we have seen weighed 1050 grams. The greatest length from tip of nose to tip of tail was 51 cm.; the majority are between 40 cm. and 45 cm. in length.

Before the traffic in these animals was prohibited by law they were to be found regularly in the markets in at least some of the large cities of the Pacific Coast. Even now they constitute a staple article of diet on the tables of many who cannot afford butchers meat, and are occasionally eaten by others.

Parasites.

Fleas: Ground squirrels are usually found to be heavily infested with fleas, the commonest species being *Ceratophyllus acutus* Baker; less frequently, the *Hoplopsyllus anomalus* Baker is found. We have shown that the former of these is capable of carrying plague among ground squirrels. No experiments have been performed with the latter. Both of these fleas readily attack man under experimental conditions and indeed also under natural conditions. At one time our squirrel stock room became so heavily infested that upon going into the room one was certain to be bitten by many of the parasites. It finally became necessary to use a pulicide upon the floor in order to make it possible to enter the room without having to suffer the attacks of the insects.

Lice, mites, and ticks are also found, the latter apparently only in certain seasons, and possibly only in limited areas. Our observations on this point are not completed. Of internal parasites, a cysticercus embedded in the muscles is occasionally encountered. Round worms and flat worms are not rare in the intestine.

Transmission of Plague.

The question of how plague is transmitted among squirrels and from them to rats and to man is one to which a positive answer cannot be given at present. Under the conditions of a laboratory experiment it is very easy to transmit plague from squirrels to guinea-pigs, to rats, and to squirrels by means of squirrel fleas, and it is not improbable that the conveyance in nature is in the same way.

It is impossible to say at present just what factors tend to limit the spread of the disease among the squirrels. It seems most likely that bodies of water and a thinning out of the squirrel population due to eradication or to unfavourable environment are probably efficient in setting boundaries to its extension.

Our work has now extended over a period of more than one year and there seems to be no reason for believing that there is any seasonal prevalence of plague among squirrels.

Extent of the infected area.

In the early part of 1909 steps were taken to determine the extent of the territory in which the squirrels were infected. The work was carried on by the U. S. Public Health and Marine-Hospital Service under the immediate direction of Surgeon Rupert Blue. The field work was assigned to Passed Assistant Surgeon W. C. Rucker, who was succeeded by Assistant Surgeon Friench Simpson, while the laboratory investigations were conducted by the writer. The plan in brief was to have the squirrels shot, packed in cans, and sent to one of the laboratories where they were submitted to a careful post-mortem examination for the purpose of detecting evidence of plague infection. In this manner, the work has been carried on over an area covering many thousand square miles. About 150,000 squirrels have been examined, and a small number of other animals, rabbits, field-mice, brush rats, etc. Up to September 1st, 1910, four hundred and two infected squirrels have been found, and one infected brush rat.

The infection has been found in ten counties in the State, and it is regarded as not improbable that in time others will be found to be infected.

One may obtain a better idea of the enormous volume of work necessary to complete the investigations when I state that in one case over 8000 squirrels were examined from one county (Monterey) before any infection was discovered. In other counties, infected animals were found in the first shipment sent to the laboratory.

The great bulk of this work has been carried on in the Federal Plague Laboratory at San Francisco, a smaller portion being done at the branch laboratories in Oakland and in Los Angeles. In the case of the latter, the work was entrusted to an assistant, not a physician, who had been thoroughly trained in the recognition of the gross lesions of plague in squirrels. When this man found lesions that he regarded



Fig. 1. Federal Plague Laboratory, San Francisco, California.

Buildings in foreground ; to extreme right, Bacteriological Laboratory, to extreme left, building for inoculated animals, in centre, rodent dissecting room.



Fig. 2. Federal Plague Laboratory, San Francisco, California.

with suspicion, the suspected tissue was sent to the laboratory at San Francisco and there submitted to the usual inoculation tests. This plan was found to work very satisfactorily.

In the early part of the work, the diagnosis in the case of every infected squirrel was verified by a complete bacteriological examination; that is *B. pestis* was isolated and identified in pure culture. Later, on account of the large volume of work, it was found impracticable to do this and only those cases were bacteriologically verified that came from new localities, usually from a different farm. When 20 or 30 infected rodents were received from one ranch, obviously it would have served no particular purpose to have verified all of them bacteriologically. It is perhaps needless to remark that each squirrel received had been tagged to show the locality from which it came and that a careful system of checking was adopted in order that each infected rodent might be properly located.

Human Cases.

The cases of plague in man, with perhaps one exception which will be mentioned later, that have been traced to squirrel infection, presented no points of especial interest. The symptoms were not to be distinguished from those seen in plague originating from rat infection. The cases have all been of the bubonic type and there is this rather remarkable fact; with but two exceptions, in each of the nine cases of which the writer has personal knowledge, the primary bubo has been located in the axillary region. It seems probable that this is due to the fact that in plague of squirrel origin the infection is usually contracted from handling infected rodents, while in the case of plague derived from rat infection, it most frequently happens that the infection enters through the lower extremities, since these parts are more exposed to the bites of fleas.

The one case of especial interest to which reference has been made was reported in detail by Wherry and the writer⁽³⁾. The case began as an ordinary, fairly severe attack of plague. The acute symptoms however subsided and for a time it seemed probable that the patient would recover. Later he fell into a pyaemic state, and died on the 16th day of the disease. The post-mortem findings were unusual for plague. There were necrotic foci in the kidneys and in the liver, a large number of caseous nodules up to a walnut in size in the lungs, and multiple caseo-purulent lymph glands. In other words, the post-mortem appearances were those of subacute plague.

One other case seems worthy of mention. The facts were as follows:—

Late in the summer of 1908, a boy living in the city of Los Angeles was taken ill with symptoms of such a nature that the suspicions of the attending physician were aroused. The case followed the usual course of bubonic plague. The primary bubo was in the right axilla. A bacteriological examination of the fluid aspirated from this bubo showed a pure culture of an organism that gave all of the characteristic reactions of *B. pestis*. The case was a severe one, but the patient eventually recovered after a prolonged illness, during which several of the superficial glands suppurated.

No case of plague had ever been seen in this part of the State, and, so far as was known, there had never been any plague among the rodents of the city or its surroundings. The history however gave a clue to the origin of the infection. About five days before the boy became ill, he had picked up a ground squirrel for the amiable and charitable purpose of taking it to where it could get water to drink. The animal appeared sick, and it must have been very ill to have submitted to being captured by hand. The squirrel bit the boy on the right hand, and, as before stated, he became ill about five days later and developed a right axillary bubo. Whether the bite infected the boy, or whether ectoparasites from the squirrel got on him and infected him, we do not know. The squirrel escaped and its fate is unknown. While the boy was sick, a dead squirrel was found at a point within 100 yards of the place where the boy was when bitten. The squirrel was shown by bacteriological examination to be plague infected. Exterminative measures were undertaken, during which several thousand squirrels were killed and examined, but no case of squirrel plague was found beyond the one previously mentioned. The sick squirrel and the dead one were found a short distance from the switching yards of the railroad which connects Los Angeles with San Francisco. At the latter place a rather widespread epizootic of plague had prevailed among the rats a few months before. The most reasonable way to explain the squirrel infection in Los Angeles is by assuming that an infected rat was carried from San Francisco, and this rodent in some way caused a small outbreak among the squirrels in the territory adjacent to the railroad yards. This case has been related somewhat in detail as it was almost of the nature of a laboratory experiment. It is the only one in which there is a history of a bite by a squirrel.

Three other cases of plague in squirrel hunters have come under my

observation. All of these men shot and handled squirrels on farms on which infected squirrels were known to exist. In one case, symptoms appeared three days after contact with the squirrels; in the others, after an interval of five or six days.

Since the sanitary authorities have been in a position to ascertain the facts in relation to squirrels, no case of plague has occurred in the State, except those that have developed at points where ground squirrel plague existed.

All of the cases of plague in man have been submitted to careful bacteriological examination, and in each instance the plague bacillus has been recovered.

The question of the mortality among persons infected from ground squirrels may be answered as follows: Of the nine cases of which I have personal knowledge, five died. This number is of course too small to be used as a basis of comparison, but one is probably justified in saying that there is at least no striking difference between the mortality from plague of squirrel origin and that of rat origin. I shall in another place make mention of the virulence for laboratory animals of the bacillus isolated from infected squirrels, and from persons presumably infected from squirrels.

Lesions of Natural Plague in Ground Squirrels.

The lesions of plague in squirrels resemble more closely the changes found in plague in the guinea-pig than those present in rats and mice. They present a wider variation than those found in other animals. We may have nothing to excite suspicion beyond a small purulent focus in a lymph gland, but when this is submitted to the test of inoculation into a guinea-pig, the results show that plague infection is present. On the other hand, we may have very extensive necrotic changes not only in the majority of the superficial glands, but in the internal organs as well.

The lesions may be described briefly as follows ⁽⁴⁾:

A *bubo* is present in about four-fifths of all cases. One or more glands may be affected. Those in the inguinal region are most frequently involved; next in frequency came those in the cervical region, and finally those in the axilla. The *bubo* is occasionally surrounded by a haemorrhagic area; usually it is not. There is sometimes a gelatinous infiltration in the surrounding tissue, but this is not common. In many cases, changes in the tissue around the gland

involved are almost entirely wanting. The contents are usually either blood stained, rather dry, and distinctly necrotic (caseous) or in the form of a yellowish tenacious semifluid mass (purulent). The former is probably but an earlier stage of the latter. Upon microscopical examination buboes that are distinctly caseous are generally found to be swarming with characteristic bacilli, while the purulent ones usually show no organisms at all, or none that bear any close resemblance to the pest bacillus.

The spleen. This organ is usually enlarged and frequently contains caseous or purulent foci.

The liver. Lesions are found less often in the liver than in the spleen, but when they do occur they are of the same nature as those seen in that organ.

Lungs. Lesions in the lungs are found rather frequently. They fall into two classes: first, a rather diffuse gray consolidation which may affect a whole lobe or a whole lung; second, disseminated purulent or caseous areas varying in size from a pea to a pin-head.

From our experience it would seem that one is justified in speaking of the following types of cases.

Acute cases. A bubo is practically always present. There is often some haemorrhage into the periglandular tissue. The gland structure is replaced by a reddish or salmon coloured, rather dryish, friable mass. The spleen is generally very much enlarged and is dark in colour. Pest-like bacilli are abundant.

Subacute cases. Here there are caseous or purulent lesions in one or more of the internal organs, liver, spleen and lungs. There is usually, but not invariably, a bubo. Pest-like bacilli may be numerous, but usually are scarce or absent.

Residual abscesses. A large number of cases are found in which there is only a purulent lymph gland or an enlarged gland showing a few purulent points. Pest-like bacilli are rarely found. These are looked upon as cases that are in process of recovery.

Bacteriology.

Smear preparations: In only about one-fourth of the cases will stained smears show the presence of a sufficient number of cocco-bacilli, characteristic in size and shape, to justify one in attaching much importance to them in making a diagnosis.

Cultures: From ground squirrels presenting lesions of the nature mentioned above, we have isolated an organism giving all of the cultural

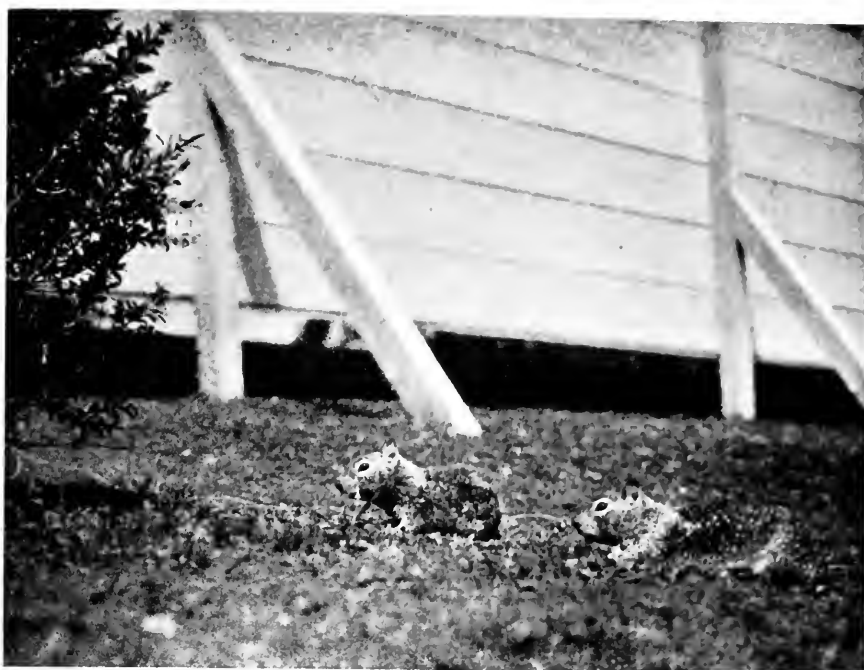


Fig. 1. Live Ground Squirrels (*Citellus beecheyi*).

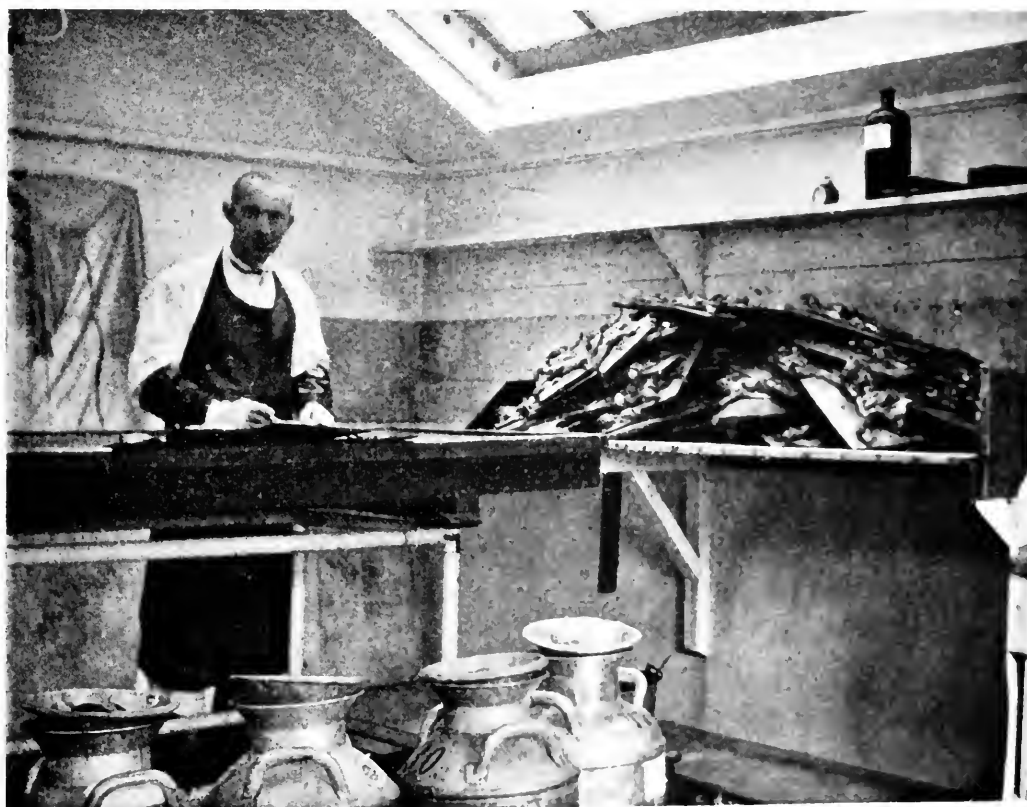


Fig. 2. Federal Plague Laboratory, San Francisco, California.

Laboratory assistant dissecting squirrels. Note cans in foreground. Squirrels are shipped to laboratory in these cans.



reactions of *B. pestis*. Study has failed to show that it is in any way different from the bacillus isolated from cases of plague in man or in rats. It is not considered necessary to go into the cultural reactions here, but I may say that the growth on agar is in the form of translucent, sticky, drop-like colonies; in broth, flocculi are formed, with a precipitate later. On salt agar, characteristic involution forms are developed.

Pathogenicity: The organism isolated is virulent for rats, mice, guinea-pigs, and rabbits, and produces the usual lesions of plague in these rodents. The virulence in some cases is as high as that of strains isolated from natural plague in rats and in man; in others, it is lower. White rats inoculated with small doses nearly always die on the third, fourth or fifth day, while guinea-pigs generally die on the fourth, fifth, sixth or seventh day, occasionally later.

We have tested the protective power of anti-pest serum against the bacillus isolated from squirrels, and from human cases believed to have been infected from squirrels. As a result of the experiments with the bacilli isolated from three human cases and from five squirrels, we have shown that the anti-pest serum (imported) invariably protects against the infection.

A Plague-like Disease of Squirrels.

In the search for plague among ground squirrels, a very interesting disease has been observed. It is characterized by a caseous bubo (sometimes haemorrhagic) and necrotic foci in the spleen and liver. The lesions would certainly lead one to suspect plague infection. Further, the resemblance to plague is heightened by the fact that guinea-pigs inoculated from these squirrels die between the fifth and eighth days with lesions that are remarkably like those of plague. The cause of the disease, however, is but feebly pathogenic for rats. This fact, together with negative results of stained smear preparations and of cultures, readily differentiates it from plague. No etiological agent has yet been discovered.

Significance of Plague among Squirrels.

This subject will be considered first in relation to the cases of plague in man that may be expected to arise from the presence of the disease among the squirrels. The experience in the past has been that the largest number of cases of plague attributed to squirrel infection in

one year was three. This makes it evident that there is no special cause for alarm on this ground. It is of course quite possible that cases have escaped observation, or have inadvertently been reported under another diagnosis.

As was pointed out two years ago by Wherry⁽¹⁾, and as I have had ample opportunity to observe myself, rats and squirrels may live in close association. Recently, Converse⁽⁵⁾ has reported the presence of ground squirrels and rats in the same burrows in the suburbs of San Francisco. It would seem that an important problem of the squirrel plague situation is the prevention of the infection of the rats in the cities of the country.

Another danger is the infection of other rodents in or contiguous to the area of squirrel plague infection. We have found one infected brush-rat (*Neotoma*) and it is possible that in time other wild rodents will be found to be infected.

Whether the epizootic among the squirrels will die out in time is a question that no one can answer at present. It is worth noting that in certain of the counties that are known to be infected, many of the healthy squirrels are quite resistant to laboratory inoculation with *B. pestis*, while the squirrels from localities in which no infection has been found are almost uniformly susceptible to infection.

Extermination of Squirrels.

Farmers have long carried on a warfare against ground squirrels on account of the damage the rodents do to crops. The usual procedure is to poison the animals with strychnine, or some other toxic agent that is administered on grain; another method is to treat the burrows with bisulphide of carbon. Both methods are efficient, and each one has a definite sphere of usefulness; the former in dry weather, the latter during the wet season when the moist ground will "hold the gas."

Eradication on a large scale. The Legislature of the State of California has passed a law requiring land holders to exterminate squirrels and other rodents on their premises and providing a penalty for failure to do so. Plans are being matured for enforcing this law, and it is believed that within two or three years the rodents may be eradicated or at least so reduced in number as to cause the extinction of plague among them.

Squirrel extermination for protection of cities. As there seemed to be some probability that the rats in the cities of Oakland and Berkeley adjacent to the squirrel infested territory might become

infected, a campaign having for its object the extermination of the squirrels in the territory immediately surrounding these cities was inaugurated by the writer. The work has been reasonably successful. It was found that while it was simple enough to poison all or practically all of the squirrels in the territory, there would be an influx from the periphery to take the place of those that had been destroyed. An interesting point that developed in connection with this work was the discovery of a large focus of infection within less than a mile of the city of Berkeley.

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OBSERVATIONS UPON THE NATURAL HISTORY OF EPIDEMIC DIARRHOEA.

By O. H. PETERS, M.D., D.P.H.

Mansfield.

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I. INTRODUCTION.

THE following observations upon the Natural History of Epidemic Diarrhoea were made in Mansfield during the summer and autumn of 1908. The fact that at the time the writer was engaged in preparing a paper—to which the present paper is to some extent complementary—upon the epidemiological relations of season and disease, lent special interest to the enquiries regularly made from the Health Department of this town into the circumstances attending fatal attacks of diarrhoea. Early in the season a more than usually extensive enquiry was made into one of these fatal attacks in an area where an outbreak of diarrhoea appeared to be spreading outwards from a group of old privy-middens. To test how far the condemnation of the latter was justifiable another area was taken on the other side of the town, where the houses were newly built and provided exclusively with water-closets; and records, collected by house-to-house visitation, were obtained of all cases of epidemic diarrhoea, whether non-fatal or otherwise, occurring in these localities. The enquiries thus begun were afterwards extended so as to embrace two fairly large districts, a chance of doing this being provided by the opportune postponement of the addition to the department of certain work of inspection which had been impending at the beginning of the summer. These districts were several times revisited and scattered observations were also made throughout the other parts of the town. During 1909, while there was no opportunity of making extended observations, there were valuable opportunities during the course of the routine inspections of the summer of testing and re-testing the principal results obtained during 1908.

The two areas thus systematically studied had a combined population of more than 2000, living in 413 houses. Of these houses 391 were each visited on several occasions during the season of 1908, from May to November, and 390 separate attacks of epidemic diarrhoea were recorded. Full details were obtained as to dates of attack, age in-

cidence and age constitution of the population, milk-supply, sanitary arrangements, proximity to stables and fly nuisance. Information as to meteorological and other relevant matters was also obtained.

The special value of this mass of raw material lies in the fact that it represents, not a record of scattered and unrelated cases, but a *complete and consecutive account of all diarrhoeal attacks* occurring in a large area and *throughout the course of the whole epidemic season*. A reference to the literature of the subject shows that substantial statistical records of diarrhoeal *sickness* are unfortunately still very scanty. They appear to be confined to those derived from the following sources: cases applying for medical relief at Poor Law establishments and hospitals: a few records of cases occurring in private medical practice; and scattered cases gathered by the Public Health staff from around the neighbourhood of fatal attacks. In three cases notification has been recently adopted. It would appear however from observations to be referred to later on, that only a small percentage of the total cases, with a disproportionate number of infants to adults, voluntarily seek medical relief, and by so doing afford an opportunity for notification to the Health Authorities; which suggests that, in records gathered in that way, allowance must still be made for a considerable margin of error.

The material was wholly collected by myself. The obscurity in which the clinical and pathological entity of the disease is wrapped makes the gathering of such data a peculiarly difficult matter. And the trained discrimination necessary in deciding what to retain and what to reject makes it almost essential that, for purposes of research, the collection of such data should only be undertaken by a medical man. In the course of the subsequent analysis and preparation of the data for the following paper, it was seen from the outset that, though a little reconsideration of the original verdict might be perfectly legitimate in regard to many details collected, and greatly assist the force and clearness of argument, yet, owing to the difficulty of knowing where to stop such revision, a rigid rule should be made, wherever doubt existed, to regard whatever conclusion was arrived at at the time of collection of the data as final.

General observations were also made in all parts of the town, including 13 other districts of a few score houses each evenly distributed throughout the Borough, and these will be referred to as support to conclusions drawn from the two large districts, the examination of the data from which will form the main body of this

paper. Some confirmatory observations, as already stated, were made in the season of 1909.

Description and comparison of the two districts from which data were mainly obtained. The two districts, one triangular and the other quadrilateral in shape, can be compared in Charts I and II, App., where they are drawn to the same scale. They are situated on opposite sides of the town, almost due East and West from its centre, and the centre of one district is distant about a mile from the centre of the other. They exhibit as complete a contrast as could be obtained in the matter of site and sanitary circumstances, as the following brief résumé suggests:

The Triangular Area.

Houses: Many old houses (sandstone); also many newer ones (brick), from 8 to 12 years old.

Rows of shops along the borders of the area.

Sanitary details: Many old privy-middens and pan (pail) closets. Greater part w.c's.

Several stables.

Site: Well-manured Allotment Gardens formerly occupied site of newer houses.

Well-drained site (sandstone). Situated half-way up the slope leading from the centre of the town. Mean altitude 365 ft.

Exposed to North and West winds only.

The Quadrilateral Area.

All new houses (brick); none older than 4 years; much building still going on.

Almost wholly residential.

All w.c's.

Practically no stables.

Formerly clean meadow land.

Well-drained site (sandstone and clay). Situated on the high ground surrounding the central part of the town. Mean altitude 420 ft.

Exposed to all winds, particularly from East.

Data as to the town of Mansfield.

Site: an upland situation, between the plains of the Trent and the plateau of Derbyshire. It lies at the junction of the New Red Sandstone with the Magnesian Limestone.

Altitude: about 250 feet above ordnance datum in the basin-shaped depression at the centre of the town, rising in outlying parts of the borough sometimes to over 500 feet.

Industries: formerly cotton-doubling, hosiery, and agriculture. The collier population is now rapidly increasing.

Population has increased rapidly and is given as follows: 15,900 in 1891; 21,400 in 1901; 32,500 in the middle of 1908. The people of the town are a young, healthy stock, with a high *birth rate*, 33·6 in 1908; and a relatively low *death rate*, 13·8 in 1908.

Infantile Mortality Rate: 151 per 1000 births for the ten years 1898–1907, and 137 for 1908; against respective rates of 142 and 121 for England and Wales for the same periods.

Diarrhoea Mortality Rate: is not officially given, but calculated from a total of 104 deaths in the last five years, 1904–8, it was 46 per 1000 deaths, and 68 for 1908. The respective figures for the neighbouring city of Nottingham calculated from the death rate were 59 and 42.

Provision of Sanitary Conveniences, etc.: from data in the Health Report for 1908, the houses were in that year provided as follows:

Midden-privies in	662	houses.
Pan (pail) closets in	665	„
w.c's in	5202	„

The midden-pits are generally provided with doors, and used as ashpits. The town is sewered throughout and has a good water supply.

II. A STATISTICAL STUDY OF AGE INCIDENCE, PREVALENCE, FATALITY, ETC.

The statistical matter here presented is not of more than moderate proportions. However, the two large districts chosen originally for the contrast they presented in sanitary and other relevant matters, have been divided into sections, so that the returns for the various sections, and again the totals for each district, can be compared. Such comparisons have been included wherever space allowed. An appeal to internal evidence can thus be made. Again, it may be pointed out that the thorough disentangling and fairly complete weighing up of the numerous complex influences affecting the distribution of the disease, which has been effected throughout the paper, has provided the means of dealing very satisfactorily with quantities of data of quite microscopic proportions. Thus the writer would feel confidence in a demonstration of the main facts from material provided by the triangular district alone.

1. *Age Incidence.*

Incomplete returns from a number of houses have been excluded altogether. A number of possibly doubtful cases have also been omitted in preparing the “corrected table” (Table II *b*); but the difference thus produced is however practically negligible, and as it is not practicable to duplicate the various tables the data of the “uncorrected tables” have been used throughout the paper.

TABLE I. *Proportions affected of Parents* and Children in Houses attacked with Epidemic Diarrhoea within the two districts. "Uncorrected Table."*

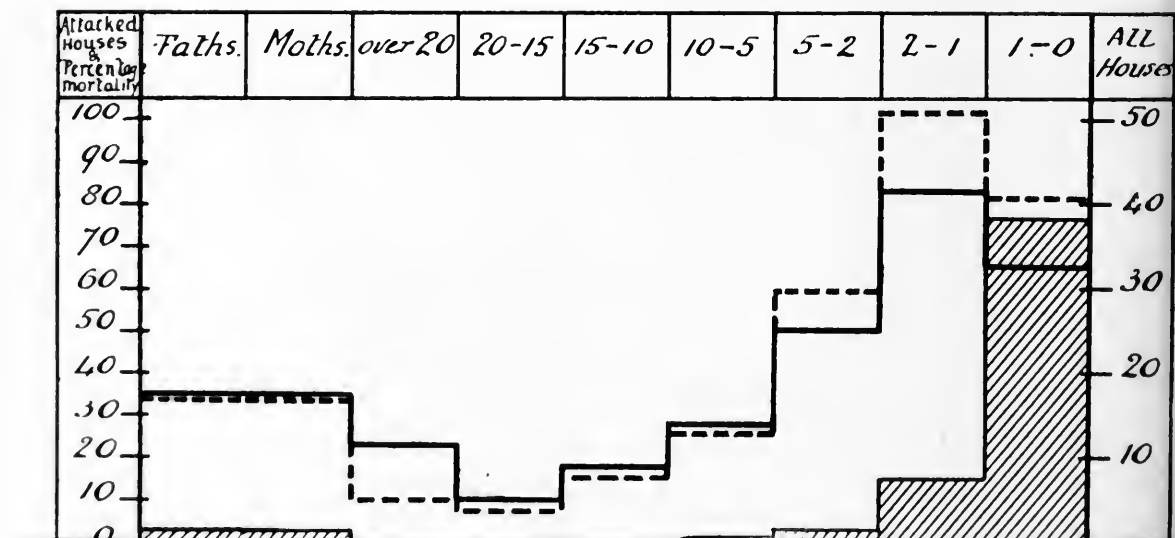
	Fathers*	Mo-thers*	20 years & over	20-15	15-10	10-5	5-2	2-1	1-0	All persons
All persons	184	187	46	77	110	146	119	36	51	956
Persons attacked	67	67	11	9	20	40	61	30	33	338
Percentage incidence of diarrhoea.	36	35	23	11	18	27	51	83	64	35

TABLE II a. *Proportions affected of Parents* and Children in All Houses within the two districts. "Uncorrected Table."*

	Fathers*	Mo-thers*	20 years & over	20-15	15-10	10-5	5-2	2-1	1-0	All persons
All persons	375	387	145	164	232	280	196	52	73	1905
Persons attacked	67	67	11	9	20	40	61	30	33	338
Percentage incidence of diarrhoea.	17	17	7	5	8	14	31	57	45	17

* Throughout the paper, "Fathers," "Mothers," and "Parents" include married persons both *with* children and *without* children, unless otherwise stated.

CHART A. *Age Incidence in Diarrhoea. The Sickness data is according to the above Tables I and II. The distribution at the various ages of every 100 Diarrhoea Deaths at Mansfield is also shown, calculated from the 138 deaths occurring during the five years 1904-8: it corresponds closely with that of London for the corresponding period.*



Diarrhoea incidence in Attacked Houses only — ; in All Houses ----- ; Mortality ■.

The most important points in the above chart and tables are the following :

(1) The result is somewhat at variance with the conception of the age incidence formerly held with regard to diarrhoea; and the attack age incidence is evidently quite different from the mortality age incidence.

(2) The totals under two years of age in the mortality table usually form about nine-tenths of the total number of diarrhoea deaths in the whole population, but in the observations here dealt with cases of sickness under two years form considerably less than one-fifth of all.

(3) Although the high attack rate amongst infants is the most striking feature of the age incidence, yet adults are not so very much less affected than young children: the proportionate incidence upon parents, children over five, and children under five, was roughly as 38 : 20 : 60 in attacked houses, and as 30 : 15 : 60 in all houses. The susceptibility of children decreases with increase in age to a minimum at the period 15—20 years; after which, even apart from the bringing up of a family and the resulting contact with susceptible children, there is perhaps a general increase up to extreme old age.

(4) Though the greatest fatality and the greatest attack incidence both fall upon children under two years of age, yet within that age period a great dissimilarity is found in regard to these matters: the mortality incidence is greater under 12 months than above that age, the incidence on the second year to that on the first being as 1 : 5; but the attack incidence in the second year is greater than that in the first year in the proportion as 5 : 4. As regards the comparative susceptibility of the youngest children, it is also worth noting that between

TABLE II b. "Corrected Table." Proportions affected with Epidemic Diarrhoea in All Houses within the two districts, separately and taken together. Also proportions of houses attacked.

			Fathers	Mothers	20 years and over	20—15	15—10	10—5	5—2	2—1	1—0	All persons	Proportions of houses attacked
All persons	360	373	133	160	230	273	190	49	67	1835	391
Attacked persons	66	67	9	8	20	37	58	27	30	322	175
Percentage Incidence	Triangular area		19	18	5	5	6	9	30	65	46	16	45
	Quadrilateral area		17	17	8	5	11	19	30	42	42	18	44
	Both areas		18	17	6	5	8	13	30	55	44	17	44

two and three years of age the attack incidence has not yet fallen below that of the first year of life ; the fatality has, however, decreased at this age to a very insignificant figure.

With regard to the results obtained by other observers, Ballard (1887-8) discusses the relative numbers of cases occurring under five years and over five years. At Islington he found it roughly as 6:1, and at Leicester 2·2:1. He inclines to the view that the former more truly represents the correct ratio, and gives his reasons for that belief (*ibid.* p. 26), which may be compared with the remarks on p. 751 of this paper. At Mansfield the ratio was as 2:1, almost the same as at Leicester, and from the manner in which the former data were collected, and from the above mentioned remarks, the writer regards this ratio as the more correct.

Owing to pressure of space the reasoned statistical enquiry as to age incidence has been abridged ; only the conclusions arrived at, and the tables on which they are founded, being given.

(a) *Variations in incidence at different ages.*

The rise to a high incidence in older persons is to some extent affected by, but is probably not altogether determined by, their more frequent and closer association with susceptible infants (cf. Tables III (a) and (b), and V). There was some appearance of an abrupt rise in incidence at the age generally corresponding to the beginning of family life ; but not a great deal of evidence as to a steady increase in susceptibility to diarrhoea sickness up to extreme old age, as suggested by the mortality data.

TABLE III a. *Some details as to the distribution of infection amongst Parents and Children in the two districts.*

Families collected into groups according as the eldest child was	Parents affected, as a percentage of all parents	Parents affected in pairs, as a percentage of all parents attacked	Families where both parents remained unaffected, as a percentage of all families	Families where all the children remained unaffected, as a percentage of all families
Above 10 years	16·7	42	40	27
Under 10 years	17·7	50	51	12
Total	17	46	46	20
25 and upwards	7	50	50	50
25—20	15	41	41	27
20—15	21			
15—10	16	43	36	23
10—5	21	50	45	15
5—2	11	60	60	13
2—0	18	44	56	6

TABLE III b.

Amongst 145 " children " over 20 years, 11 persons, or 7 % were affected.
Amongst 17 uncles, aunts, and lodgers, 5 persons, or 17 % were affected.
Amongst 15 grandfathers and grandmothers, 5 persons, or 33 % were affected.

N.B.—Perhaps the latter, when they had not been attacked, were sometimes likely to have been forgotten, at the visit of inquiry.

The obviously high intrinsic susceptibility of young children is no doubt largely influenced, as regards which particular trimestral period is to present the maximum incidence, by the method of feeding (cf. p. 673).

At the age period of lowest incidence, 15 to 20 years, there is still a moderate amount of intrinsic susceptibility. This is evidenced in many ways : e.g. 30 % of these cases were first cases, and a large number were the only cases occurring in their respective households (see also Table VIII).

Incidence on sex is approximately indicated in Table XI. There does not appear to be a great difference in incidence upon the two sexes (cf. Ballard, 1887–8, p. 29).

(b) *Statistical evidence for increased transmission of the disease amongst the members of a household owing to the close associations of family life ; demonstrating the occurrence of infection from a personal source.*

(1) The very high degree of *multiple infection occurring in households* is evidenced in Table IV.

TABLE IV. *Showing the amount and degree of multiple infection in all the 174 attacked households in the two districts. Alternate tabulations are presented below.*

Families containing	Number of families	Percentage of all attacked families	Families containing	Number of families	Percentage of all attacked families
1 case	91	51	1 or more cases	174	100
2 cases	44	25	2 „	83	47
3 „	23	13	3 „	39	22
4 „	8	4·5	4 „	16	9
5 „	4	2·2	5 „	8	4
6 „	0	0	6 „	4	2
7 „	3	1·7	7 „	4	2
8 „	1	·5	8 „	1	·5

(2) The marked influence of *association with susceptible children*, in increasing the incidence upon parents and other members of the family, is evidenced:

Firstly, in the results exhibited in Table V below :

TABLE V. *The percentages attacked, of "parents" and children, in all houses containing children, according as to whether they do, or do not, contain infants (under 2).*

	"Parents" in family groups, taken according as the age of the eldest child was					All children above 2 years	Degree of dirtiness of attacked houses
	25—15	15—10	10—5	5—0	All parents		
In houses <i>with</i> infants (under 2).	23	20	23	14	19	17	55
In houses <i>without</i> infants (under 2).	18	15	17	12	16	16	52

Note the consistent nature of the greater affection of parents where babies are present, as displayed in the various family age-groups, questions of age susceptibility being eliminated : also the still more important fact that the higher incidence was more marked in parents than in other members of the family, owing unquestionably to the closer association of the former with young children.

As differences in dirtiness of households (cf. also Table XVIII) and in the number of children were not apparently sufficient to explain the facts noted, this table must be taken as a most important demonstration of personal transmissibility of diarrhoea infection.

The number of attacked children, per attacked house, other than infants, was much greater in houses not containing infants.

The inclusion of houses not containing children made no important difference.

Secondly, in the higher incidence upon parents in the "2—0 group" than in the "5—2 group," Table III *a*. There was a greater number of affected infants in the first group.

Thirdly, in the abrupt rise in incidence noted, from unmarried persons over 20 years (Table II) to the youngest group of parents, whose average age is only about 26 years.

Fourthly, in Table III *a*, the tendency of both parents to be together affected increases at lower ages, as if on account of more frequent exposure to some common source of infection.

Fifthly, in the study—in the following section—of the frequency with which young children are responsible for the introduction of infection into a house.

Sixthly, in instances to be given later (cf. p. 701) of actual infection of parents from children.

(3) An examination of the effect of *the close association of married life* upon the mutual affectability of parents, and of the influence of children upon one another, gave no definite results beyond reaffirming the already noted high degree of multiple infection occurring amongst the various members of the household.

(4) *The influence of parents upon children* calls for special mention, in view of the high incidence the former are subject to and of their habitual association with young infants in whom the deplorable mortality of the disease mostly occurs. Under the next heading it will be shown that in accordance with their high susceptibility, the parents play a considerable part in the introduction or first development of infection within the family.

(c) *Upon what members of the family rests the chief responsibility for the introduction of infection.*

(1) The *number and susceptibility* of the inmates of a house were found, as might be expected, to determine, other things being equal, the degree of liability of that house to attack. Thus, houses containing the most susceptible units, children under two years, were found to have been proportionately more frequently affected than other houses (cf. Tables VI and VII).

Again, when the houses were arranged in successive groups according to the age of the youngest child, a gradually decreasing incidence was found in correspondence with the increased age and lessened susceptibility of the children (cf. Table VII).

The parents, the next most susceptible units after young children, are however necessarily excluded as a comparative factor, from their being common to most households; the influence exerted by the youngest part of the household thus practically alone holds the field as a differential factor, and most important distinctions are thereby produced.

The greater the number of children in a family the greater is the liability of the household as a whole to attack (cf. Tables VII and XXI): and this result naturally follows, independently of the fact that houses with large families are also dirtier.

(2) An enquiry into *first cases*, i.e. as to the frequency with which the various members of the family were the first to introduce or to

TABLE VI. *Showing the proportionately greater incidence upon the houses containing infants (0—2) than upon the other houses.*

The first part of the Table indicates the proportions of attacked and unattacked houses amongst those containing and amongst those not containing infants per 100 houses in each district and in the combined area.

Districts	Houses containing infants (0—2)		Houses not containing infants (2—0)		Total houses
	Attacked houses	Unattacked houses	Attacked houses	Unattacked houses	
Triangular	26	10	24	40	100
	36		64		
Quadrilateral	17	9	30	44	100
	26		74		
Both areas	21	9	27	43	100
	30		70		

The second part of the Table indicates the proportions of attacked and unattacked houses per 100 houses containing infants, and per 100 houses not containing infants, in each district and in the combined area.

Districts	Houses containing infants (0—2)			Houses not containing infants (0—2)		
	Attacked houses	Unattacked houses	Total houses	Attacked houses	Unattacked houses	Total houses
Triangular	72	28	100	38	62	100
Quadrilateral	65	35	100	40	60	100
Both areas	69	31	100	39	61	100

Total number of houses containing infants (0—2)=119: all other houses=272.
Cf. also the remarks as to the mass-action of houses containing infants, p. 651.

TABLE VII. *Showing how the incidence upon houses varies according as they contain children of more or less susceptible ages. The incidence is also seen to vary according to the size of the family. The data as to dirtiness are included for purposes of correction. Cf. Table XXI.*

	All houses containing infants under 2	All houses containing children from 2 to 5 yrs., but none under 2	All houses containing children 5 yrs. or over, but none under 5	All houses containing children 10 yrs. or over, but none under 10	All houses containing children
Percentage of houses attacked	69	47	33	30	49
Percentage dirtiness of houses	57	55	44	41	52
Percentage attacked { Houses with 3 children & less	71	38	30	29	49
Percentage attacked { Houses with 4 children & more	64	54	35	35	
Percentage dirtiness { Houses with 3 children & less	52	48	44	41	52
Percentage dirtiness { Houses with 4 children & more	64	62	46	42	

TABLE VIII. *Showing what proportion, as a percentage, of all cases at each period were the First Cases in the season to occur within their respective households.*

Districts	Father	Mother	20 yrs. & over	20-15	15-10	10-5	5-2	2-1	1-0	All persons
Triangular	50	65	42	60	25	41	56	61	45	53
Quadrilateral	65	54	100	25	33	47	51	58	76	55
Both areas	58	58	63	44	30	45	54	60	57	54
Both areas (aver- ages of 3 groups)	59			40			56			54

TABLE IX. *Showing that the tendency to figure as First Cases varies as the season advances, the tendency at some age periods decreasing in intensity, and in others increasing. The distribution in the various age groups is shown of 100 First Cases occurring in each half of the season. The data are from the two districts.*

	Fa- thers	Mo- thers	20 yrs. & over	20-15	15-10	10-5	5-2	2-1	1-0	Total first cases
First half of the season	24.3	22.5	6.3	1.8	3.6	6.3	17.1	11.7	6.3	100
Second „ „	16.8	20.7	3.7	1.2	3.7	14.2	19.4	5.1	14.2	100
Percentage occurring in 1st half	56			38			47			50
Percentage occurring in 2nd half	44			62			53			50

TABLE X. *Showing the variation in incidence (expressed as percentages of all persons at the different age periods) as the season advances. The data are from the two districts.*

	Fathers	Mothers	20 yrs. & over	20-15	15-10	10-5	5-2	2-1	1-0	All persons
First half of season	30	35	52	13	23	16	42	83	39	32
Second „ „	33	31	28	8	10	33	56	76	71	33
First „ „	33		52	18			50			32
Second „ „	32		28	20			63			33
First „ „	34			18			50			32
Second „ „	31			20			63			33

develop infection, showed that this was generally in proportion to the degree of their susceptibility. Perhaps adults, it may be from their wider daily peregrinations, are a little more liable than others to become introducers of infection at the beginning of the season; and it is probable that an important part is played by children in the second year of life, who were the "under ones" of the preceding diarrhoea season, in handing on the disease from that season, and lighting it up again in their respective households (cf. Tables VIII, IX and X). It is important to note also the greater tendency of certain age-groups to develop attack or to figure as first cases in the first than in the second half of the season. The early marked incidence upon ages 1—2 is in great contrast to the delayed attack of infants under one year (cf. Tables IX and X), the probable interpretation of which has just been given above.

TABLE XI. *Incidence on Sex. The sex-distribution of all persons attacked in the two districts. Not complete.*

Districts		Fathers	Mothers	20 years & over	20—15	15—10	10—5	5—2	2—1	1—0	All persons
Triangular	{ Males	32	—	2	2	1	9	17	6	8	77
	{ Females	—	32	2	2	5	8	13	11	11	84
Quadrilateral	{ Males	35	—	5	3	4	14	15	6	5	87
	{ Females	—	35	2	1	6	11	15	7	7	84
Both areas ("Uncorrected")	{ Males	67	—	7	5	5	23	32	12	13	164
	{ Females	—	67	4	3	11	19	28	18	18	168
Both areas ("Corrected")	{ Males	66	—	5	4	5	23	31	10	13	157
	{ Females	—	67	4	3	11	16	26	17	15	159
(Grouped)	{ Males	66		14			54		23		157
	{ Females	67		18			42		32		159

2. *Prevalence and Fatality.*

The very high incidence of diarrhoea throughout the districts examined was noted with some surprise. It was thought probable that for one district at least the conditions were such as to render it practically a diarrhoea-free district. The question might well be asked—Is the wholesale incidence of the disease here noted to be regarded as more or less usual in towns which are generally recognised as a little more than moderately affected with diarrhoea? Since comparisons between towns must at present depend wholly upon the death rate, it would be instructive to ascertain what ratio the total number of cases usually bears to the number of deaths, and whether that ratio is at all constant. It will

appear that the proportion of non-fatal cases to fatal cases is probably much greater than that generally stated.

In the two districts, amongst the 338 cases or 407 separate attacks in the season, there were only two fatal cases. The distribution of these was as follows :

	All cases		Fatal cases
	"Uncorrected"	"Corrected"	
Triangular area	166	156	2
Quadrilateral area	172	166	0
Combined area	338	322	2

Case Mortality .59 %

One other fatal case occurred in the western part of the triangular area, properly speaking however outside the area and amongst the houses, mostly shops, which were not visited.

It should be noted that even with this total of three deaths occurring within an area of the dimensions of the triangle, that district must be considered to have had rather more than its full share of deaths, since no area of similar size in the town had so many. On the other hand, the triangular and quadrilateral areas were no more severely affected with diarrhoea cases than many other parts of the town where observations were made. In the following season of 1909, certain parts of both districts which had a notable incidence in 1908 were again found to be almost as heavily affected, although 1909 was a cooler year with a very much smaller diarrhoea death rate. We are thus led to believe that there was nothing exceptional in what was found as to the great prevalence of cases, absolutely, and also relatively, to the deaths. Taking the lowest estimate, by dealing only with the corrected total for the one district—the triangle, in which the deaths occurred, counting the three deaths and allowing a few cases for the unvisited areas around the margin, there would be at least 60 non-fatal cases to every fatal case. Taking the visited houses of both districts with the two deaths occurring therein, there were 161 cases to every death.

An average of about 100 cases to every death, whatever the results of future observations may be, would appear to be the only reasonable estimate to be deduced as regards the relation of sickness and mortality. If this be accepted and the mortality be multiplied by 100 to give the total cases of sickness, a rather high figure for the latter, that is, as regards our preconceptions of the disease, would be generally obtained.

The 34 deaths from epidemic diarrhoea and enteritis during 1908 at Mansfield would mean 3400 cases of diarrhoea in a population of 33,000, or that 10% of the whole population were attacked. In the neighbouring city of Nottingham, in a diarrhoeal year such as 1899, with 600 deaths, the 100:1 rule would mean 60,000 cases of diarrhoea in a population of 260,000, or 23% of all. This seems almost incredible, and it is probable that the ratio between sickness and fatality varies widely in different years and again in different towns. What statistics of sickness and mortality there are at present available suggest this; and the connection between mortality and sickness in diarrhoea is, moreover, a particularly loose one, a matter which will be specially referred to later on (cf. pp. 628 and 719). As regards Mansfield, however, during 1908, the writer saw no reason to regard 3400 cases, or 10% of the population attacked, as an over-statement of the prevalence of diarrhoea.

Enquiry into this question was made in a very thorough way, for besides the triangular and quadrilateral districts, there were 15 other localities, scattered over the whole town, where I made fairly extensive observations, to be referred to later on. In addition enquiries were made around the 34 fatal cases. Again, in visiting cases of the notifiable diseases which were unusually abundant in the autumn, or in calls made for other purposes, enquiries as to diarrhoea were also made, perhaps on more than 100 occasions. From this it will be understood, when the limited area of the town is considered, that I was able to obtain an intimate knowledge of the movement and dimensions of the epidemic in every part of the town, and that there were few streets even where I was not able to make more than a mere guess as to the precise degree of diarrhoea prevalence. The absence of mortality in the large quadrilateral area was by no means an exceptional case. In six other areas where I found diarrhoea to be very prevalent no deaths were recorded. Again, in several other areas foci were found where almost every house was attacked. The third area (Chart III, App.) is a sufficiently remarkable illustration of this. In short, there was every reason to believe that the suggested affection of 10% of the population did not by any means overshoot the mark. In the two large districts it must be remembered that as many as 18% and 17%, respectively, of the population were affected. Judged by mortality, 1908 may be considered to have been a year of fairly high prevalence. The average number of deaths for the five years ending 1908 was 27; and 1906 and 1908 had the greatest number, 34 deaths each.

3. *Variations in Age Incidence and Fatality with the Progress of the Season.*

(1) The variations in age incidence and in the tendency of different age-groups to figure as first cases, as the season advances, has already been referred to at the end of Section II, 1 (c) (cf. Tables IX and X, and Ballard, 1887-8, p. 40 *et seq.*).

(2) Ballard (1887-8, p. 20) found that the "ferocity" of an epidemic increased as the season advanced, as judged by the shortness of the time in which it kills its victim. At Mansfield the case-mortality appeared to increase towards the end of the season, thus agreeing with the somewhat late maximum affection of infants under 12 months (cf. Tables IX and X). The data with regard to this point are of course too meagre to base any conclusions upon, although since children under one were affected rather later than persons at other ages, the maximum mortality might also have been expected to fall later than the maximum prevalence of all cases. They may however be quoted as follows: the outbreak in the quadrilateral was apparently as late as most local outbreaks in the town.

	Prevalence in 4-weekly periods ending				
	July 4	Aug. 1	Aug. 29	Sept. 26	Oct. 24
Cases in Quadrilateral area	12	47	73	42	19
Deaths in Town ...	1	1	8	11	9

III. CLINICAL FEATURES, IMMUNITY, ETC.

1. *Special Clinical Features; Diagnosis.*

The precise value of data as to attacks of diarrhoea, and of the conclusions drawn from them, will depend largely upon the degree of reliance that can be placed upon the *diagnosis* made at the time of their collection: and as regards the cases recorded in the above tables, some question naturally arises as to whether they were all undoubted attacks of epidemic diarrhoea. The characteristic features of epidemic diarrhoea, as regards the history of attack, are the occurrence of *diarrhoea, abdominal pain, vomiting, more or less well-marked depression, a tendency for the attack to extend for a longer period, and to take upon itself a more definite entity—so as to come to be regarded by the patient in the light of a real illness, than in the case of simple diarrhoea of a non-infective type.* As regards differentiation from the non-infective

type, in a large number of the latter there is simply looseness of the bowels without further accompaniment; as in diarrhoea following slight digestive disorder, the administration of laxative substances, or over-indulgence in fruit.

The clinical picture of the specific affection is not however always complete, and several of the typical features may not be elicited. But a history of diarrhoea and just one other of the above-mentioned signs would, in certain circumstances, raise strong suspicion as to the infective nature of the attack. Of the latter signs, severe abdominal pain is one of the commonest in occurrence, and in consequence one of great diagnostic value, while of equal value in this respect is the characteristic and profound depression. Vomiting and prolongation of the attack are of course also important. As none, however, of the above signs are exactly pathognomic of the complaint, we must, as in other infectious diseases, look to clinch the diagnosis, in doubtful cases, to the circumstances in which the case has occurred: that is, as to the presence of the diarrhoeal season, and the occurrence of other cases in the same or neighbouring houses, especially if also closely related in point of time. It must be noted that the symptom of diarrhoea is not essential to the clinical picture: it is not constantly present.

Ten instances of the latter kind were incidentally noted, most of which had pain and vomiting or other symptoms: two cases have been abstracted from the data which the writer was disposed to include as instances of epidemic diarrhoea, in which severe abdominal pain was the only symptom. In the first instance, a father was doubled up with severe pain and felt sick, but did not vomit; five days afterwards his child, aged 3, developed a complete and typical attack. The second instance was that of a married couple without children; the wife had a severe attack of abdominal pain lasting two days, felt sick, and was constipated, but did not vomit; six days after the beginning of her attack the husband developed a complete and typical attack. In both of these instances there were at the time typical cases of the disease amongst the neighbours, and other suggestive circumstances. The two commonly mentioned symptoms, convulsions and change of colour in the stools, were of comparatively subordinate interest where four-fifths of the histories were not concerned with attacks in infants. There was of course every degree of mildness as well as severity in the attacks.

As regards the existence of more than one specific disease, some of the evidence obtained appeared to suggest that this matter was worthy of consideration, but no definite conclusions were arrived at.

As the collection of data proceeded it became increasingly evident that it was not only desirable, but the only correct procedure, to throw the net rather widely than otherwise, and not to be misled by the light opinion of the attack held by the people themselves, or by the trivial causes to which they assigned it. *There could be little doubt but that practically the whole of the cases of the type collected were "epidemic," and probably also "infective" in character*, since they were practically non-existent at the beginning of the season, and decreased regularly as the temperature fell, while they were constantly related to other cases in the same or in an adjacent house. Experience taught that not too much notice was to be taken of "teething" and similar popular explanations, as evidence of a negative kind: a little careful enquiry would frequently reveal the presence of an undoubtedly specific attack of diarrhoea. Again, many apparently simple diarrhoeas in young children were found to suddenly develop in a very dangerous manner, so that the writer was forced to the conclusion that it was advisable not to regard lightly any history whatsoever of looseness of the bowels in infants during the diarrhoea season. Owing, however, to the great frequency with which digestive disturbances are known to occur in infancy, apart from specific diarrhoea, it might still be argued that little reliance should be placed upon the data relating to that period. To this it may be replied that either we must reject this conservative tendency to reduce the proportion of infective cases in infancy, or relinquish the other old conservative belief as to the much greater proportion of infants infected than adults; for amongst adults the cases were always well marked enough to leave little doubt as to their right to be included amongst specific attacks. Further, the large amount of spread from infants to other members of the family must receive due consideration in this connection.

A discussion of *the popular conception* generally entertained *as to the nature and cause of the disease* is not only theoretically interesting, but is of some practical importance in view of the education of public opinion which must eventually be undertaken in this matter (cf. p. 757). As suggested above, the general tendency is to place it amongst the list of perfectly natural and trivial occurrences. Constipation on the one hand, and diarrhoea on the other, are regarded as disorders suitably adapted for home treatment, and not requiring special medical advice. The idea of infection is of course never entertained, and in laying about for a cause it is customary to seize upon the first suggestion that comes to hand. In infants it is invariably the teeth; in adults, since it cannot be the teeth, it is fruit; and of the different kinds of the latter, plums

for preference. In the early summer, when plums are not available the cause is generally referred to strawberries. Amongst the causes assigned to demonstrably typical attacks of epidemic diarrhoea were medicine, currants, chocolates, fruit, teething, heat and cold. It is true there seems to be a generally recognised obligation to name a cause for the attack, but having discharged that duty in the manner above indicated, and generally it appears with great mutual satisfaction to all concerned, patients and friends lapse into complete indifference upon the matter. Moreover, this not incorrectly sets forth the attitude of the general public, educated and uneducated alike, to what is in reality a very great scourge and a great sanitary reproach to the community at large.

It is necessary to insist upon certain matters of practical importance arising out of this popular indifference and complete unconsciousness to the actual existence of such a widespread and economically important disease as diarrhoea. The first relates to the great difficulty in obtaining histories of diarrhoea: the first enquiry is generally met by a negative reply, even only a few months after an attack; either the attack has already passed out of memory, or the fact is not grasped, that the insignificant occurrences recalled can possibly be the subject of inquiry. *An inexperienced enquirer, who unsuspectingly neglects constant, patient, and tactful cross-questioning, will certainly miss the greater part of the cases in the houses canvassed.* Nurses and medical men are themselves prone to pass over the specific nature of the complaint, and to regard as negative in a specific sense, histories such as *e.g.*, that of being "subject to diarrhoea." The occasional negative observations as to spread in children's hospitals may possibly be found to be explained in this way (cf. note at foot of p. 706). Personally the writer would not rely upon his own recollections as to a previous negative history of epidemic diarrhoea.

It might be mentioned incidentally that in both adults and children there are a large number of attacks of great severity: so much so that it seems sometimes difficult to accept the suggestion that the attacks are on the whole more violent in warmer climates than with us. Many of the seizures in adults were of a severe choleraic type, with sudden onset, and intense prostration, accompanied by attacks of fainting, so that it sometimes seemed impossible that a fatal termination would be averted. In a large number of cases strong working-men were confined to bed for a fortnight or more; 12% of all cases, as stated before, sending for medical advice. No very complete figures as to confinement in bed were however collected.

Other features of the disease not so common as the above are the passage of blood in some severe cases: a few of the attacks were of a markedly dysenteric type: jaundice was noticed in four cases, all in the quadrilateral district; two were related, as regards place and time, to each other and to typical diarrhoea attacks. Ballard (1887-8, pp. 15 and 18) mentions fugitive rashes, and refers to the diarrhoea occurring in some scarlet fever outbreaks: none of these rashes were met with; on one occasion however the spontaneous remark was elicited with regard to the diarrhoea prevalence, "What a lot of sore throat there is with it!"; moreover in six families, during the season, there was a history of sore throat associated in the same person with attacks of diarrhoea. In two of these cases bacterial swabs of the throat were examined, but none but ordinary throat organisms were found. The possibility of their being instances of "drain throat," or mild complicated cases of the other throat affections, could not of course in so small a number be satisfactorily determined.

As regards the unsatisfactory and confusing titles conferred upon the disease: of the different combinations of the epithets "diarrhoea," "enteritis," "gastro-enteritis," "epidemic," "infective," and "zymotic," perhaps the most complete are the terms "zymotic gastro-enteritis," or "infective gastro-enteritis." But a title which purports to be a name, and nothing more, without reference to symptomatic or anatomical associations, as the name "cholera" has come to be regarded, would be, especially in the present state of our knowledge, by far the most satisfactory: and incidentally it must be confessed that, to one in daily close contact with the disease, the old title of diarrhoea, "English Cholera" or "Cholera Nostras," is the most satisfying one of all; for the clinical features of the disease are so peculiarly those of a "minor cholera."

2. *The Incubation Period.*

In a number of cases it was found possible to obtain facts as to the probable length of the incubation period, and this appeared to be frequently from 6 to 30 hours, although quite possibly it is sometimes longer. A history often obtained was that of exposure to infection on one day, followed by an attack on the day after; and less commonly, exposure to infection during the day with a sudden seizure during the same night. Bruce Low (1887-8, p. 127) describes four outbreaks of what closely resembled epidemic diarrhoea, and undoubtedly was

so in one instance, in which the disease appeared to be directly communicated from person to person, and had an average incubation period of from 10 to 12 hours: in a number of cases, however, the attack developed on the day after exposure to infection. It might be supposed that, owing to the greater chance of exposure to such a risk, infection is most often contracted during the daytime; in which case, if the length of the incubation period happened to be about 12 hours, a larger number of attacks would occur by night than by day. This hypothesis was to some extent borne out by the facts: the probably correct time of onset was collected in 32 instances, and of these 18 began between 6 p.m. and 6 a.m., and 20 began between 12 p.m. and 12 a.m. The incubation period appears from this to be nearer 18 than 12 hours in length, if exposure to the greatest risk of infection be supposed to occur between 6 a.m. and 6 p.m. Infection might of course have been contracted as long as an entire 24 hours, or several times that period, before this.

Another method of arriving at the solution of the matter is by noting that the reaction of deaths to temperature occupies from ten days to a fortnight. Since the average length of fatal attacks is sometimes nine days (cf. Table XIII), to which about two days must be added for registration, it follows that the incubation period of fatal attacks is generally less than three days.

3. *Duration of Attacks. Acute and Chronic Varieties.*

The duration of attack varies with age, and directly with the degree of susceptibility to attack, as indicated in Table II. Acute and chronic cases merge gradually into one another: there is apparently no clinical distinction to be made between them.

4. *Recurrence of Attack in the same Individual.*

(a) *During the same season.*

13% of all cases had 2 or more attacks during 1908.

1%	„	„	„	„	3 attacks	„	„	„
1 case only had					4 attacks	„	„	„

It was difficult to say whether many of these second attacks were not merely remissions of the first, owing to the smallness of the interval appearing to separate them. Such recurrent attacks merge

imperceptibly into chronic attacks with remissions, which are very typical of chronic diarrhoea. Recurrent attacks also, generally speaking, vary with age, and are commonest amongst most susceptible persons.

TABLE XII. Duration of attack of cases at each age period.

	1 week & under	1—2 wks.	2—3 wks.	3—4 wks.	er wks.	Total number of attacks	Average duration of illness in days	Number of attacks from which data are derived
Fathers	88	8	2	2	—	100	5·0	70
Mothers	86	9	3	1	1	100	5·2	75
25 & over	100	—	—	—	—	100	4·5	2
20—25	100	—	—	—	—	100	3·4	10
15—20	100	—	—	—	—	100	3·1	9
10—15	96	4	—	—	—	100	3·2	23
5—10	85	9	2	4	—	100	5·4	46
4—5	89	11	—	—	—	100	4·9	17
3—4	82	12	—	—	6	100	6·4	16
2—3	76	6	9	6	3	100	8·3	33
1—2	55	21	6	9	9	100	11·7	33
0—1	60	23	2	8	7	100	11·3	40
All ages (including Uncles, etc.).	82	10	3	3	2	100	6·3	394

TABLE XIII. Duration of attack, under two years of age, compared, in fatal and non-fatal cases.

	1 week & under	1—2 wks.	2—3 wks.	3—4 wks.	Over 4 wks.	Total	Average duration of illness in days
152 “deaths” (Blackburn) ¹ ...	64	24	7	2	3	100	9·0
100 “deaths” of “children” (Manchester) ² (93 were under 18 months).	50	25	8	5	12	100	?
73 “cases” (Mansfield) (non-fatal cases)...	58	22	4	11	5	100	11·5

¹ Greenwood, *Blackburn Health Report* for 1906.
² Niven, *Manchester Health Report* for 1905.

TABLE XIV. Cases at each age period exhibiting multiple attacks in the same season, expressed as percentages of all cases at each age period.

Fathers	Mothers	25 up.	25—15	15—10	10—5	5—2	2—0	Under 5				All ages
								5—3	3—2	2—1	1—0	
7	13	—	11	10	12	16	15	9	24	20	12	13
10								18				

(b) In different Seasons.

The recollection of previous diarrhoea attacks soon fades from the popular mind. Thus nearly half of previous attacks remembered occurred in the last four seasons. Of the total persons in two selected groups, *i.e.* of (1) those who had a definite history of previous attack, and (2) those who were definitely stated to have had no attack; 59% belonged to the first group.

5. Immunity; natural and acquired.

The question of acquired immunity will be considered from evidence discussed in the paragraphs immediately preceding.

Recurrence of attack in the same season. On the one hand, it must be admitted that the percentage of cases displaying this feature is fairly high; on the other hand it might be urged that the greater part of the secondary attacks followed closely upon and were really not disconnected from the primary; and with regard to this fact it might be recalled that certain of those diseases conferring the highest degree of immunity have a very protracted and interrupted period of immunisation. Thus typhoid fever with relapses may be said to consist of a series of separate attacks. In scarlet fever, a considerable percentage of cases develop a second attack within six to eight weeks of the onset of the first one. As regards diarrhoea, about 75% of the second attacks followed within six weeks of the end of the first one, very possibly, in most cases, through the persistence and lighting up of the primary infection. Due importance must also be given to the fact that 86% of cases appeared to be immune from a second attack; although, considering the plentiful distribution of diarrhoea cases in the two districts and particularly in certain foci, it might well be assumed that most attacked persons were exposed to infection on several subsequent occasions during the season.

Recurrence of attack in different seasons. There was a history of previous attack in about half. Considering therefore that in practically the greater number of cases the history of former attacks was forgotten, and recalling also the numerous facts already given as to the wholesale prevalence of the disease (Sect. II, 2), it might be safely concluded that, in these mining and manufacturing towns of the Midlands, most of the working class population probably have several attacks of epidemic diarrhoea during their lifetime.

Duration of attack might also be supposed to have some relation to the question of immunity. And in perfect accordance with this it was found that both the duration of attack and the tendency to recurrence of attack in the same season varied directly with the degree of susceptibility to attack, all three being most marked in young children. The correspondence of the duration of attack in Table XII with the degree of susceptibility of different age periods shown in Table II is remarkably exact, and incidentally is a striking testimony to the sufficiency of the data for a demonstration of the former point. The minimum in both cases is in the 15 to 20 years age period. It might therefore be said that at this age period the body shows greatest resistance, or the quickest reaction of immunisation; expressed in popular everyday phraseology, it throws off the disease best at this age.

Fatality and severity of attack, there is no doubt, had also some relation to the question of immunity. Fatality of attack varies with the degree of susceptibility, being greatest in infancy and extreme old age; and immunity has already been connected up with susceptibility and duration of attack. Fatality in diarrhoea however does not depend only on the one factor, severity of attack, but also apparently very largely upon the physical resistance of the patient. Thus the effect of the greater severity of attack in infancy and possibly old age is magnified many times over by the low physical resistance at these age periods. It cannot, however, be definitely stated that the severity of the symptoms apart from their mere duration was actually greater in infancy than, for example, in the 15 to 20 years age period: there were of course no means of accurately testing this point. On the contrary, from the descriptions of the patients themselves, there seemed no reason to believe that at the latter age the attacks were much less severe during the few days through which they lasted; and the type generally met with was a short and rather sharp attack, frequently with violent diarrhoea, pain and vomiting, and limited to about two or three days. The older patients, in accordance with their greater susceptibility, appeared to be perhaps more severely affected than the above, the attack being also sometimes considerably prolonged.

In summing up the above evidence as to immunity in diarrhoea, it must be remembered that, while the percentage of multiple attacks in the same or in different seasons was undoubtedly high, the chance of repetition of attack was much greater than that generally obtaining in any of the other infectious diseases, owing to the extraordinary and wholesale prevalence of the disease in question; a prevalence which, in

the season, has no parallel with that witnessed in other infectious diseases, except in very extraordinary manifestations of the latter—where however the occurrence of second attacks, again, is usually common enough. That there is a moderate degree of immunity is evidenced not only by the large percentage of cases that, notwithstanding the above-noted ubiquity of infection, contrive to escape a second attack during the season; and by the suggestion in the epidemic curve of speedy collapse from exhaustion of susceptible material (cf. Sects. VII, 3 (b), II, 2 and 3); but also by the very great resistance offered to the disease by certain age-groups, particularly at 15 to 20 years and thereabouts, even in houses where infection is present. What is the precise nature of the great immunity evidenced at this age, whether natural or mostly acquired, it is difficult to decide. Finally, it is interesting to note that the variation of immunity with age in typhoid fever is exactly the opposite of that in diarrhoea, the ages of greatest and least susceptibility in the former being respectively the periods of least and greatest susceptibility in the latter.

6. *Some Clinical Aspects of the Mortality.*

Certain wide differences have been already pointed out between the morbidity age incidence and the mortality age incidence in diarrhoea, and the almost complete limitation of mortality to the first one or two years of life has been contrasted with the very general distribution of cases of sickness amongst all ages. But further than this, so large a part is played in this mortality by non-specific factors, such as actual pre-existing disease, or debility and bad nutrition related to defective social conditions, that the attack of diarrhoea almost comes to be regarded as rather an incident or accidental complication of the fatal illness; merely administering the finishing touch, like acute pneumonia in some other diseases. And thus the differences in diarrhoea, studied from the point of view of cases of sickness on the one hand, and of fatal cases on the other, are so great that they almost require the separate recognition accorded to two different diseases. Relatively, one may say, in a free generalisation, which must not be taken too literally—diarrhoea itself, *i.e.* as regards morbidity, is a disease of health (cf. p. 748): but as regards mortality, it is a disease of ill-health. Even a little over-statement of this matter, at the present time, should be rather beneficial than otherwise; for it is only too apparent on following out the literature of the subject, how facts related almost exclusively to, and derived almost exclusively from, the mortality data, have over-

coloured the true conception of the real disease. With the present rapid increase of sickness data, some readjustment of perspective must be looked for.

Two sets of observations, which afford a basis for the foregoing remarks and are well worth following out at length, must here be briefly mentioned.

Ballard (1887-8, p. 43 *et seq.*) found that in 332 fatal cases 57.5% "had been either weakly from birth, or had been subsequently weakened by disease antecedently to their fatal diarrhoea attack." Niven, in the *Manchester Health Report* for 1904, pp. 179-181, found that in one lot of 111 diarrhoea deaths of children under 12 months, 75 had had "poor health" prior to the fatal attack, and 31 were said to have been in "good health," although the perfect soundness of all in the latter division is seriously doubted. The weakness was due to the following causes: "failed to thrive" (14 cases); "previous illness" (12), tubercle being frequently suspected; "previous diarrhoea" (17), tubercle suspected in half of these; colds and exanthemata (11); indigestion (11); 56 of these were probably insufficiently nourished. Only four of the 111 were being fed on the breast, the bulk of the others being fed on fresh milk and condensed milk. From the examination of another lot of 98 deaths (1905 Report, pp. 116-7) similar conclusions were also drawn. The results are summed up as follows: "It is very clear that the previous condition of the infant is one of the chief determining factors of fatal diarrhoea."

An examination of Mansfield mortality data also gave similar results. Perhaps one of the most important facts to bear in mind is that the change from mother's milk to cow's milk or other food, generally made between three and twelve months of age, is a physical feat on the part of the digestive organs only accomplished with difficulty; and any disturbing cause, such as slight chronic or acute inflammation in the digestive tract, may be sufficient to render this at once impossible of safe accomplishment. Deserted by its digestive allies, the child readily succumbs to the strain of any exhausting disease, such as diarrhoea, which may supervene.

IV. SOCIAL RELATIONS.

The death rate from epidemic diarrhoea has been found by various observers to vary widely amongst different classes of the community, the better classes being relatively immune. It is not unlikely, although in

view of the peculiar relationship of the mortality to sickness it does not necessarily follow that the same rule holds good with respect to the general prevalence of non-fatal as well as fatal attacks of the disease. Should this be so, a comparative study of the different conditions obtaining in the various strata of society should give many useful hints as to the influences controlling the prevalence and spread of the disease. The following include perhaps the chief differences which might be supposed to distinguish the habits and conditions of life amongst the better classes in this connection: (1) greater general cleanness: (2) a much smaller degree of closeness of personal contact: (3) greater care in the protection and preparation of food. It must be remembered that in the better class districts the common infectious diseases, generally, are recognised to be much less prevalent than amongst the artizan and labouring classes, presumably owing to the influence of the first two factors above mentioned. The first and third factors are dealt with in the two following sections in relation to diarrhoea. The second as to the effect of varying degrees of intimacy of personal intercourse will receive consideration in the present section.

The question as to the extent to which the *middle and upper classes* are subject to diarrhoea sickness is a very interesting one. No direct observations were made on this point, but from casual remarks gleaned, the impression was received that the disease was not at all rare, and perhaps more common amongst the middle classes than is generally believed, particularly when their houses are located amongst or have their rear premises bounded by, small cottage property from which infection may pass to them. For many reasons, already discussed (p. 622), the presence of the disease amongst these classes does not come to the knowledge of the public; the cases being ignored or their details suppressed; the deaths also occurring perhaps comparatively infrequently. One can imagine, moreover, that diarrhoea might not have so secure a footing amongst the better class households, owing to the very solid opposition presented by the high degree of cleanliness there maintained; but that in years of special prevalence such areas may be subject to serious inroads of the disease from the humbler quarters of the town, in which it has been able to establish itself in the completely endemic form.

Socially, the population of both districts might be said to belong exclusively to one class—artizans, small tradespeople, and colliers. The wages were regarded as comparatively high, and likely to attract workmen of a high degree of physical fitness. The people were con-

stitutionally of a strong and healthy stock, well-nourished, well-clothed, and prosperous; poverty being practically absent, and drink also as a cause of poverty; there being a tendency to excess rather in eating than in drinking. Thus the factors of drink, poverty, and insufficient food and clothing which complicate the study of diarrhoea in the slums of large cities were absent, and the only difference of a social kind was as regards dirty and careless habits of living and in the care of food: but it should be noted that the differences amongst the various household units with respect to these factors were apparently as great as between each of the various social classes taken as a whole. Thus spotless cleanliness and an unexceptionable family ménage were constantly found side by side with gross filth and neglect. This shows how misleading general statistics as to prevalence in widely distant localities might be if collected without full knowledge of all the qualifying factors concerned. The quadrilateral area was notable as presenting, in passing from the S.E. to the N.W. corner, a gradual gradation from faultless household cleanliness and care in food, and comfortable living, to the other extreme. A general comparison of the two districts has been given in the introduction. That comparison will now be extended by the addition, as occasion offers, of further details in this and the following section.

1. *Houses, Rents, etc.*

The houses were of two storeys, and mostly arranged in long terraces. The general interior arrangements, which were on a very uniform plan throughout the districts, were as follows (see Chart III, App.): There were three bedrooms upstairs and four rooms downstairs; the latter including a front sitting room, frequently not used, a kitchen generally used also as the eating and living room, a small scullery, and a small pantry, usually provided with fair means of lighting and ventilation. Bathrooms and additional bedrooms were found where the rents were above 6s. to 7s. From the kitchen and eating room a door led back into the scullery, and another, closely adjacent and to the side, opened immediately into the backyard. In the newer houses the rear part of the building was continued back behind the scullery, and was divided into two compartments to form the coal house and w.c., both communicating separately with the backyard. The rents were generally a good indication as to the condition and habits of the householders. They ranged from 4s. to 7s. in the triangle, and 5s. 6d. to 10s. in the

quadrilateral. In the triangle more than three-quarters were between 5s. 3d. and 6s. 2d., with only six houses above the latter figure; in the quadrilateral nearly two-thirds were between 5s. 6d. and 6s. 3d., and three-quarters were between 6s. and 10s.; a third of the latter being above 8s. The higher rents in the latter district were not determined by the number and size of the rooms, but by the fact that the houses were newer, had larger gardens, were further from the centre of the town, and in a higher and more breezy situation. The nine houses in the triangle (Nos. 131—139) at 4s. to 5s. were very old

TABLE XV. *Showing the House-Rents per week, and the number of houses at the various weekly rents in the two districts.*

Districts	4/-	4/3	5/3	5/6	6/-	6/2 & 3	7/-	7/6	8/-	8/- to 10/-
Triangular	9	4	10	64	84	5	5	1	—	—
Quadrilateral	—	—	—	53	12	68	24	—	14	38
Both areas	9	4	10	117	96	73	29	1	14	38

TABLE XVI. *Showing the weekly House-Rents in the various streets of the two districts.*

Triangular Area.

α Street—5/3.

β Street—5/6 to 6/2.

γ Street—East side 6/-; West side 5/6.

δ Street—5/6 to 6/-

κ Street—Nos. 131—139, 4/-.

Nos. 140—154, 5/- to 6/-.

μ Street—6/-.

Nos. 155—158, 7/-.

Quadrilateral Area.

π Street—North side 5/6; South side 6/3 & 6/-.

ρ Street—North side 6/-; South side 79—86, 6/-; 87—93, 7/-; 94—107, 8/-.

τ Street—5/6.

σ Street—North side 7/-, Nos. 171—173, 8/-.

South side 131—153, 5/6; 184—187, 6/-; 174—183, 183—213, 8/- to 10/-.

stone terraces containing sometimes only three rooms to the house, and though generally kept in a cleanly condition, were an inferior class of property. With the possible exception of the latter, however, there were no representatives in the two districts of the type of slum property found in large cities. Other details of the houses and of their situation are given in the Table on p. 606. They were mostly new; that is, built within the last ten years, and in accordance with recent bye-laws. Generally speaking, the housing may be said to have been good, both as regards lighting, ventilation, and in most other important sanitary

details. The differences in rent did not serve to mark very great differences in housing in the individual districts. As regards the majority of the houses, there was only the difference of a shilling in the rents of the different houses of each district. The houses over 8s., in the quadrilateral, in σ Street, were, comparatively, of a very superior class.

2. Occupation.

In both districts the people were mostly artizans or colliers receiving comparatively high wages. Factory hands and unskilled labourers were practically absent in the quadrilateral, and were not numerous in the triangular area. The number of mothers employed away from home was quite negligible. As regards the possible influence of occupation upon the chances of infection, it was remarked in the section (II, 1 (c)) dealing with age incidence that sometimes the fathers appeared to bring infection home with them, perhaps from their work. Table XVII sets out, in the form of fractions, the numbers affected with diarrhoea over the total numbers employed at each trade. The figures are not complete for all houses in the triangle, nor for the southern half in the quadrilateral. They are, however, large enough to give some idea of the status and occupation of the people dealt with, and to yield an instructive comparison.

TABLE XVII. *Showing the Occupation of the head of the family in the various households of the two districts.*

The proportion of households attacked in each case is indicated by fractions, showing the number of houses attacked over the whole number under each occupation.

	Triangle	Quadril.	Total		Triangle	Quadril.	Total
Colliers ...	27/39	18/26	45/65	Agents ...	—	4/4	4/4
Grocer ...	3/4	1/1	4/5	Traveller ...	—	1/1	1/1
Baker ...	0/1	1/1	1/2	Contractor & builder	1/2	1/1	2/3
Butcher ...	—	0/1	0/1	Clerk ...	1/1	1/1	2/2
Storekeeper ...	0/1	0/1	1/2	Railway employees:			
Painter ...	1/1	1/2	2/3	Enginedriver, fireman	—	5/11	5/11
Carpenter, joiner ...	1/2	1/1	2/3	Porter, guard ...	1/1	1/2	2/3
Fitter ...	—	2/2	2/2	Shunter, signalman	1/1	2/3	3/4
Foundry, moulding...	2/4	1/2	3/6	Police ...	—	1/1	1/1
Motor works ...	—	3/4	3/4	Mill: hosiery ...	2/5	3/6	5/11
Brickworks ...	3/3	1/2	4/5	Knitter ...	—	1/1	1/1
Tramcars ...	—	1/1	1/1	Shoe factory ...	—	1/1	1/1
Corporation workman	3/4	—	3/4	Miscellaneous ...	3/10	1/2	4/12

Totals:—Colliers 45/65 ; i.e. 69 % of such houses were affected with diarrhoea.
All others 57/92 ; i.e. 61 % , , , ,

It would appear from this Table that diarrhoea shows no limitation whatever to certain special trades and callings. In one instance, however, a special increased incidence might have been expected, viz., amongst colliers. Their special liability to typhoid has been several times noted, being due apparently to their careless habits of causing faecal pollution of the underground workings. The same might be expected to be even more marked in the case of diarrhoea, where such a practice is more a matter of sudden necessity than of carelessness. The households of colliers were affected in 69% of cases, against 61% in others; but against this may be placed the fact that colliers' houses were dirtier; and, particularly in the triangle, they included a higher proportion of houses containing susceptible infants (cf. Table XXII). A more satisfactory result is obtained by finding what proportion of fathers in the various occupations were affected. Amongst colliers 29% were attacked, and 19% in other callings. On the other hand, only 6% of colliers furnished first cases against 14% amongst all others, so that perhaps infection in uncleanly homes is most probably the real cause of the high incidence. At any rate the margin of cases is not sufficient, taking into account the incompleteness of the records, to indicate with any certainty that infection is brought home from the colliery much more frequently than from other places of employment. In other words, if much diarrhoea be brought home it is perhaps more probably due to the mere influence of personal association whilst at work, rather than to any special conditions in the work itself, apart at least from the method of disposal of the faeces.

3. *School Attendance.*

Bruce Low (1887-8) has described an outbreak of diarrhoea of an epidemic type arising from the use of a common privy at the village school. As regards personal contact, however, if the latter is of any importance at all, the effect of school attendance should receive some consideration. In diarrhoea, it is to be noted, the reverse conditions hold to those met with in scarlet fever and diphtheria, where school children embrace the most susceptible ages; for in the former disease, children of school age, particularly in the upper school, are at the time of life when most resistance is offered to infection. It is adults in their association at work who are the members of the family most likely to contract infection by contact with members of other households. Again, the incubation period of diarrhoea appears to be short and the

onset sudden and unmistakable, and sufferers must perforce remain out of school, which is not nearly so often the case with diphtheria and scarlet fever. A good many records of school attendance were taken, but no indication was obtained as to special affection of any one school. In both districts four different schools were attended, the children very often attending three or four different schools from the same family.

4. *Yards-in-common, and other factors determining closeness of human intercourse.*

Houses with backyards in common. There was a marked tendency for houses so related to be affected together, or together escape infection. Yards common to several houses, from 2 to 15 in number, were found throughout the triangle. In the quadrilateral, yards-in-common were only found in the seven houses on the north side of π St.; in the four end-houses on the south side of ρ St.; and in the 20 houses with backs adjacent, along the north side of σ St. These yards however provided little more than a narrow common entry from the street, owing to the railing off of garden plots at the back. A common entry, but of the kind providing very little opportunity of mixing of the neighbouring households, can also be made out on the charts in other parts of the same district. In this area, whatever the nature of the entry, the yards were always separately divided off; the dividing fence however was generally only three feet high with large interstices, of three or four inches, between the wooden palings; thus providing little hindrance to intimate association between the young children of neighbouring houses, and between the parents also. The practical relation of these facts to the question of diarrhoea incidence will be considered in a later section (Sect. VII, p. 689).

Structural arrangements of the back premises determining a specially close relationship between successive pairs of households.

Chart III shows the internal structural arrangements, already alluded to, typical of most of the terraced houses of the two areas. The effect of the backward prolongation of part of the rear premises deserves special consideration; the backward prolongation of two adjacent houses forming a deep recess or quadrangle, into the innermost angles of which the back entrance of each house opens. These doors, which lead directly into the scullery and kitchen, eating or living room,

thus open directly opposite each other and frequently at a distance of only 12 feet. The recess in the case of houses in the middle part of μ Street was only seven ft. wide, and sixteen ft. deep, with walls on three sides from 10 to 20 ft. high. An inner yard is thus formed, which must tend to restrict the movements of both the householders themselves, and possibly of fly-carriers, within this area; to the exclusion of their neighbours, who have to be reached by a considerable journey around the backward prolongation of the rear premises. It must also be noted that the doors of the w.c's open also opposite each other and into this area, the distance to the kitchen door of either house being also very little. The practical application of these facts will be considered in a subsequent section (Sect. VII, p. 691).

The data under Section IV may now be considered complete with the exception of facts as to personal intercourse between neighbours, and visiting between friends. Such data, if they could have been obtained, might have yielded important information, amongst other things, as to the occurrence of direct personal infection, and as to the transplantation of infection throughout a district.

V. SANITATION.

1. *Cleanliness of the household.*

The factor of household cleanliness has been frequently mentioned as probably holding an important place in the causation of diarrhoea, and it was therefore considered advisable and necessary to collect data under this heading. Marks were given, ranging from 1 to 5, to denote the degree of cleanliness or dirtiness of each home. The maximum of five indicated the greatest degree of dirtiness and untidiness, particularly as regards the leaving about of food exposed to the dirt and dust of the household. The minimum of one mark denoted that the family ménage was unexceptionable. The result of this enquiry showed that cleanliness of the home has an important relation to the comparative incidence of the disease in that it exerts a definite influence in warding off attack. It is important to record that as a means of gauging comparative dirtiness or cleanliness, the method adopted gave very reliable results (cf. p. 642). For the original data as to dirtiness Table XXVII *b* App. should be referred to.

(a) The first method of presenting the data collected is as in Table XVIII, which sets forth, amongst other things, *a comparison*

between the average dirtiness per house of those attacked and of those unattacked with diarrhoea. The marks allotted to each house have been added up and divided by the number of houses coming under each heading. The averages thus obtained have been then expressed as a kind of percentage, the maximum of five marks being equated to 100, and the others to 80, 60, 40 and 20 respectively; the equivalent of one mark being taken as 20, and not as 0, in order to facilitate the handling of the data.

TABLE XVIII. *Showing the comparative dirtiness, expressed as a kind of percentage (see text), in all households containing children, grouped according as to whether they do or do not contain infants (under 2 years), and were or were not attacked with diarrhoea.*

The houses were divided into 5 classes; the dirtiest=100; the cleanest=20.
Cf. also Table VI.

Attacked and unattacked houses			All houses		Houses with and without infants (under 2)			
Both Areas :								
Houses attacked	With infants	55	54	52	{ 57	{ 55	Attacked	Houses with infants
	Without infants	52				{ 62	Unattacked	
Houses unattacked	With infants	62	50		{ 49	{ 52	Attacked	Houses without infants
	Without infants	47				{ 47	Unattacked	
Triangular Area :								
Houses attacked	With infants	58	58	58	{ 60	{ 58	Attacked	Houses with infants
	Without infants	57				{ 65	Unattacked	
Houses unattacked	With infants	65	57		{ 56	{ 57	Attacked	Houses without infants
	Without infants	55				{ 55	Unattacked	
Quadrilateral Area :								
Houses attacked	With infants	51	50	47	{ 54	{ 51	Attacked	Houses with infants
	Without infants	49				{ 60	Unattacked	
Houses unattacked	With infants	60	44		{ 44	{ 49	Attacked	Houses without infants
	Without infants	41				{ 41	Unattacked	

Tested in this way, the comparative excess of dirtiness in attacked as against unattacked houses, expressed in the ratio 54:50, is not great, but the result will be shown later to be qualified by various factors; two important ones having relation to the presence of young children and the size of the family. The qualifying influences of association with dirty and highly attacked neighbours, and of the sharing of "yards in common," also further tend to mask all distinctions between the total dirtiness of attacked and unattacked houses.

It is sufficient however to note here the following points as regards houses containing children.

Amongst all houses, the attacked were dirtier than the unattacked as 54:50.

Amongst all houses not containing infants (under 2), the attacked were again dirtier than the unattacked as 52:47.

Amongst all houses containing infants (under 2), however, the attacked houses were actually cleaner than the unattacked as 55:62: although houses with infants were decidedly more dirty than other houses as 57:49, and these two facts were consistently observed in both districts. We might conclude, therefore, that houses containing infants were not more heavily attacked merely because they were dirtier than houses not containing infants; and that the influence upon diarrhoea incidence, dependent upon the presence of susceptible infants, is not on parallel lines with, and is powerful enough to subordinate to itself, that dependent upon dirtiness of the household. Attacked houses containing infants were, however, still dirtier than attacked houses not containing infants, in the ratio 55:52. The exceptional relations of the diarrhoea incidence to dirtiness in houses containing infants will be more fully discussed later on (Sect. V, 1 (e)), and, along with other things, the fact that breast-feeding saved a large number of infants living in the dirtiest houses of both districts from attack will be shown to satisfactorily dispose of the difficulty.

The importance of the above conclusions is emphasised by the fact that they are all borne out by the results obtained from the two districts taken separately as well as together. As regards houses without children, there was no difference in the degree of dirtiness of attacked and unattacked houses, the ratio being 37:37. But these houses were few in number, were scattered, and were practically all of one standard of cleanliness; 23 out of 28 were allotted either 1 or 2 marks, and only one was marked at more than 3. For this and other reasons it has been found convenient, and quite legitimate, to exclude them from most of these tables.

(b) A second and more striking method of presenting the data is shown in Table XIX, where *attacked and unattacked houses are compared as regards the relative numbers exhibiting the various degrees of dirtiness, as indicated by the index figure allotted to each house*. The result shows, as was to be expected, a preponderance of unattacked houses at the low indices, and a preponderance of attacked houses at the high indices; but an interesting feature is exhibited in the fact that by far greater difference exists at the end of the table apportioned to the cleanest houses, the houses in the first column exhibiting a very

special tendency to remain exempt (cf. p. 655). In comparing the relative numbers of houses attacked and unattacked under each index figure, a correction should of course be made as regards the relative percentages of attacked and unattacked houses in the district or

TABLE XIX a. Showing the number of attacked and unattacked households containing children, exhibiting the various degrees of dirtiness, as denoted by the index figure at the head of each column.

All houses containing children										
Districts	Numbers of houses which were	Indices of dirtiness					Indices of dirtiness			Percentage of houses attacked & unattacked
		1	2	3	4	5	1 & 2	3	4 & 5	
Triangular Area	{Attacked	5	20	25	11	6	25	25	17	53
	{Unattacked	6	17	20	8	8	23	20	16	47
Quadrilateral Area	{Attacked	11	26	20	9	2	37	20	11	45
	{Unattacked	21	34	16	9	2	55	16	11	55
Both Areas	{Attacked	16	46	45	20	8	62	45	28	48
	{Unattacked	27	51	36	17	10	78	36	27	52

Districts	Numbers of houses which were	Houses not containing infants (under 2)				Houses containing infants (under 2)			
		Indices of dirtiness			Percentage of houses attacked & unattacked	Indices of dirtiness			Percentage of houses attacked & unattacked
		1 & 2	3	4 & 5		1 & 2	3	4 & 5	
Triangular Area	{Attacked	10	15	6	40	15	10	11	73
	{Unattacked	21	15	10	60	2	5	6	27
Quadrilateral Area	{Attacked	23	14	6	39	14	6	5	62
	{Unattacked	50	11	6	61	5	5	5	38
Both Areas	{Attacked	33	29	12	40	29	16	16	68
	{Unattacked	71	26	16	60	7	10	11	32

The percentage of attacked houses in these Tables refers only to those houses in which records of dirtiness were made.

TABLE XIX b. Houses containing Infants (under 2), arranged into two groups according as the infant was fed on—

		Breast Milk		Cow's Milk	
		Indices of dirtiness		Indices of dirtiness	
		1 & 2	3, 4 & 5	1 & 2	3, 4 & 5
Triangular Area	{ Attacked	11	11	6	13
	{ Unattacked	3	10	0	2
Quadrilateral Area	{ Attacked	5	8	12	2
	{ Unattacked	1	8	4	2
Both Areas	{ Attacked	16	19	18	15
	{ Unattacked	4	18	4	4

districts considered: these percentages are given in the last column, the total number of attacked to unattacked houses in the two districts taken together being as 47:53. Thus, before the numbers of attacked houses under the different indices can be compared with corresponding numbers of unattacked houses the former must be multiplied by $\frac{53}{47}$, or the latter by $\frac{47}{53}$. The alteration however would be so slight that the correction need not be inserted, and on an inspection of the tables it is at once evident that it would not be sufficient to explain the differences noted.

Houses not containing infants were seen to show a still greater proportion of unattacked houses to attacked under indices 1 and 2; the contrast being greatly lessened when all houses were added together, especially in the triangle, on account of the directly opposite behaviour of houses containing infants, which have a much greater proportion of attacked than unattacked houses under 1 and 2. Thus the irregularity noted in this class of house in Table XVIII again makes its appearance.

(c) A third method adopted was to establish *a comparison between a group containing the cleanest and a group containing the dirtiest neighbourhoods* in the two districts. More striking results were thus obtained (see Table XX), depending on the fact that clean and dirty houses were not evenly distributed throughout, but tended to be respectively grouped into a number of clean and dirty neighbourhoods. This was most marked in the long and drawn-out quadrilateral area, where the dirtiest houses were grouped towards one end of the district, and the cleanest towards the other. The effect of this segregation of clean and dirty houses was to present a kind of *mass action* of cleanliness or dirtiness, apparently intensifying many times over the almost inappreciable effect of individual houses (cf. p. 654). Thus where clean houses were distributed singly amongst dirty houses, differences in liability to diarrhoea were to a great extent masked or smothered; but where they were gathered together into a large group, they might be supposed to together present a solid phalanx towards the centre of which diarrhoea appeared to have greater and greater difficulty in penetrating. Cf. π and σ Streets in the quadrilateral.

In Table XXa the two districts have been divided into a total of 33 sections: each section marking off a row or rows of houses in which there was a more or less constant tendency to one particular standard of dirtiness. The cleanest and dirtiest sections have been then respectively grouped so as to, as nearly as possible, halve the total number of houses in each district.

The result shows that, even in the triangle, where the other methods failed to elicit any great contrasts, the excess of dirtiness and diarrhoea incidence in the dirty sections over that in the clean ones is very marked. Houses containing infants were, however, also more numerous in dirty sections: but it will be shown later that this only accounted in part for the greater diarrhoea incidence (cf. Sect. V, 1 (g)).

TABLE XX a. *Contrasting the comparative dirtiness and comparative incidence of diarrhoea of the cleanest and dirtiest groups of sections of the two districts.*

The two districts have been divided into 33 sections, and the cleanest 16 are compared with the dirtiest 17 sections. For comparison, the percentage of houses containing infants (0—2) is indicated. The Table includes both houses with, and houses without, children.

Districts	"Clean sections"			"Dirty sections"			Number of sections		Number of houses included in	
	Percentage dirtiness	Percentage of houses attacked	Percentage of houses with infants (under 2)	Percentage dirtiness	Percentage of houses attacked	Percentage of houses with infants (under 2)	Clean	Dirty	Clean sections	Dirty sections
Triangular	44	45	27	68	56	44	9	10	84	98
Quadrilateral	41	37	18	53	58	34	7	7	109	100
Both Areas	42	40	22	60	57	39	16	17	193	198

b. *Showing the number of houses exhibiting the various degrees of dirtiness (as denoted by the dirt indices at the top of the columns) in Clean and Dirty Sections. Cf. Table XVI.*

	All houses containing children		Houses not containing infants (under 2)		Houses containing infants (under 2)	
	1 & 2	3, 4 & 5	1 & 2	3, 4 & 5	1 & 2	3, 4 & 5
"Clean Sections"	37	20	18	14	19	6
	57	18	55	12	2	6
"Dirty Sections"	25	53	15	27	10	26
	21	45	16	30	5	15

(d) Before proceeding to draw conclusions from Tables XVIII, XIX, and XX, the influence of the age-constitution, size, and occupation of the family upon the degree of dirtiness must first be examined. Their special bearing upon the degree of diarrhoea incidence has already been studied in Sect. II and Tables VI, VII, XXI, and XVII, to which a reference might again be made.

From general observations it appeared that throughout the districts, with the almost complete absence of domestic help, household cleanliness constantly passed through certain definite evolutionary stages as the family grew up. The home of the young newly married couple was generally very clean. In proportion as the number of children increased however, to some extent because of the inability of the mother to cope with the large increase of household work so entailed, the standard of cleanliness dropped lower and lower. With cessation of child bearing and entrance of the family into their teens an increasingly higher standard of cleanliness prevailed, probably owing to the operation of factors such as the following. The enforced activity of the mother during the years when the bulk of the family were young children, needing constant attention, has become a fixed habit, and as that burden has been gradually removed, her energies have been diverted into matters dealing more strictly with the general cleanliness of the home. To this she is also impelled by the increasing self respect of the children as they grow up, particularly of the daughters, who are now also able to assist her; the wage-earning capacity of the family is also increased and they are able to provide many extra comforts and perhaps a more agreeably situated home. As the children leave home and the parents have only themselves to care for, the acquired habits of cleanliness remain, and the home is found to be, if anything, cleaner than at any other time. These facts are consistently borne out by the data of Tables XXI and VII, where the size, age-constitution, and dirtiness of the family are fully considered. Such complete conformity of the data with generally observed and accepted facts is at the same time important intrinsic evidence of its reliability; and it is for this reason that the latter have been outlined with such full detail.

The presence of babies in the household is one of the most important factors tending to dirtiness. This is not so marked amongst young parents where one or two babies comprise the whole family, but where childbearing has continued after the eldest child has reached 15 or 20 years it can generally be inferred that the family is too large to be kept at a high standard of cleanliness. As regards the size of the family, a point of some significance was that the number of children per family was much less in the quadrilateral area: 280 per 100 families with children, against 350 in the other district: the explanation certainly did not lie in the age or physique of the parents. The people of the quadrilateral district were more intelligent, and though of the same class, a somewhat superior type to the people in the triangle.

Their small families and their more disciplined habits contributed to make their district much cleaner than the other.

The above facts should be carefully followed out in Table XXI: it is there evident that the smaller families, containing three children or less, were consistently cleaner and less heavily attacked, except in the case of houses containing infants under two, which though still cleaner were more heavily attacked. Cf. also Table XXVII *a*.

There was also great variation in the degree of dirtiness of the household according to *occupation*. It was found in the preceding section under the heading of "occupation" (cf. Table XVII) that the houses of colliers were more often affected than those of persons following all other occupations taken as a whole. The chief reason for this, however, seemed to lie in the much greater dirtiness of houses of colliers; and, particularly in the triangle, in the greater proportion of their houses found to contain infants, as shown in Table XXII. Colliers' houses were mostly confined to a few neighbourhoods in each district; which, apparently from such houses providing the dirtiest dwellings

TABLE XXI. *Showing to what extent dirtiness, and incidence of diarrhoea, vary with the size of the family.*

Only houses containing children are included here, and only those in which data as to dirtiness were obtained. Cf. Table VII, and Table XXVII *a*.

		All houses containing		Houses <i>with</i> infants (0-2), and containing		Houses <i>without</i> infants (0-2), and containing		Houses with children all 5 years or over, and containing	
		3 child- ren or less	4 child- ren or more	3 child- ren or less	4 child- ren or more	3 child- ren or less	4 child- ren or more	3 child- ren or less	4 child- ren or more
Percentage attacked of all houses		47	51	72	62	36	45	34	35
		48		68		39		34	
Percentage dirtiness of	All houses ...	48	60	53	64	47	56	44	46
		52		57		49		45	
	Attacked houses	48	62	48	68	48	58	—	
		54		55		52			
	Unattacked ,,	48	54	65	59	42	52	—	
		50		62		47			

The two areas considered separately :

Percentage attacked of all houses	Triang.	53	52	83	66	38	38	38	36
	Quadri.	42	50	63	54	33	50	31	35
Percentage dirtiness of all houses	Triang.	52	66	55	65	51	67	—	—
	Quadri.	45	50	52	61	42	47	—	—

and from their more frequently containing susceptible infants, especially in the triangle, than other houses, proved to be localities showing the highest incidence of diarrhoea. Colliers' houses in the triangle were almost limited to α and γ Streets, on both sides of each street: and in the quadrilateral to π Street and the northern side of ρ Street.

TABLE XXII. *The relations between occupation, dirtiness, and diarrhoea incidence.*

Districts	Colliers			Other occupations			Numbers of houses from which data were collected	
	Percent. dirt.	Percent. of houses attacked	Percent. of houses containing infants	Percent. dirt.	Percent. of houses attacked	Percent. of houses containing infants	Colliers	Others
Triangular	72	69	60	44	57	31	39	40
Quadrilateral	55	69	30	43	65	28	26	52
Both Areas	65	69	47	44	61	29	65	92

(e) *The irregular relations to dirtiness of houses containing infants* (under 2) also require special examination before final conclusions can be drawn from Tables XVIII, XIX and XX. In these tables, amongst houses containing infants, the dirtiness of the unattacked houses was actually greater than that of the attacked; a result so pointedly the converse of that found amongst houses not containing infants, particularly in Table XIX, as to suggest that the element of chance enters to some extent into the question. The irregularity in the former class will now be critically examined.

In the first place, as appears from Tables XXI and VII, it so happened that the greater incidence of diarrhoea in houses containing infants fell upon the smallest, and on that account the cleanest, groups of families; whereas, in houses not containing infants, it fell upon the largest families, which also proved to be the dirtiest families. These facts were evident also in both districts; and most markedly in the triangle, especially in the clean sections of the latter. Moreover, in the same area, the highly attacked yards were, if anything, a little cleaner than the yards of low incidence, while they contained a much greater proportion of infants (cf. Table XXIV). Examining these houses individually upon the chart, it appears that 7 out of the 12 attacked houses with infants in the clean sections in the 1 and 2 columns (Table XIX) occurred in the comparatively clean μ Street, with an average family of 2.7 children, and an average dirtiness of only 37. In this case then, the irregularity was founded on an irregular distribution

of infection, at least with regard to the degree of dirtiness. Another fact tending to produce irregularity is recorded in Table XXIII. The comparative percentage incidence of diarrhoea, upon houses containing infants, in the "clean" and "dirty" sections, was as 69:69; being also about the same in both triangular and quadrilateral areas. On the other hand, upon houses *not* containing infants, there was a much smaller incidence in the clean sections, which after all might have been expected, being as 31:46; and similarly in the respective districts.

TABLE XXIII. *Showing percentage of houses attacked amongst—*

	Houses containing infants (under 2)		Houses not containing infants (under 2)	
	Clean sections	Dirty sections	Clean sections	Dirty sections
Triangular Area	73	71	29	42
Quadrilateral Area	65	67	33	50
Both Areas	69	69	31	46

This naturally tends to reverse the relations as to total dirtiness of attacked and unattacked houses in the two classes of households concerned; and, should there be no differential influence from dirt, the total dirtiness of all attacked houses containing infants should be at least no greater than that of the unattacked. It would in fact be definitely a little less; there being a fraction more of incidence upon clean sections. In the triangle therefore the irregularities in question may be largely attributed to the many irregularities observed in the relation of dirtiness to diarrhoea.

As regards the quadrilateral, however, the irregular relation amongst houses containing infants, of attacked and unattacked houses to dirtiness, which was observed in both clean and dirty sections, cannot be successfully disposed of along these lines. As a matter of fact, in the quadrilateral, the attacked houses of the above class, although cleaner, in the ratio 51:60, yet had somewhat larger families than the unattacked, in the ratio 2·9:2·4; where all such houses are included. It might here be added that the absence of records of dirtiness in some houses probably did not affect the issue.

We come now however to facts as to *infant feeding* which satisfactorily dispose of all doubts upon the matter. These apply particularly to the quadrilateral, where in dirty houses (indices 3, 4 and 5) breast-feeding was ten times more common, in proportion to feeding on cow's milk, than it was in clean houses (indices 1 and 2): the proportion of unattacked

infants thus came to be much larger in dirty houses than in clean houses (cf. Sect. VI, 1). From Table XIX *b* it will be also seen that the excess of dirtiness of unattacked over attacked houses of this class could be satisfactorily attributed in each district to the large number of dirty houses (indices 3, 4 and 5) which were evidently saved from attack by the breast-feeding of infants; very little effect being noted however in clean houses.

It might be noticed here that the probable error for individual houses, in collecting the data, is large, the choice oscillating, *e.g.* between 2 and 3, or between 3 and 4; *i.e.*, between 40 and 60, or 60 and 80. The practical inference from what has been said then is that houses containing infants tend to show, individually, complete independence of dirt in developing infection; also, in the dirtiest sections, there is no greater proportionate incidence than in the cleanest, the percentages being as a matter of fact just equal (cf. Table XXIII). The greater total incidence upon clean houses is due to the irregular distribution of infection in clean sections, and of breast-feeding. Finally, it will be shown that within the attacked households of those containing babies, dirtiness still increased the incidence upon the other members (cf. Table XXVIII *b*). It may be concluded then that in the results as to houses *not* containing infants the element of chance played very little part, and that the opposite relation to dirtiness of houses containing infants may be ignored. Moreover, the number of the former class of houses was two and a half times as great as the latter, and therefore likely to yield more reliable results; and there was a constant and marked association of attacked houses of this class with greater dirtiness in all the analyses and tables presented, from Tables XVIII, XIX and XX, onwards.

(*f*) *The characteristic arrangement, in diarrhoea, of attacked houses in groups or clumps* (see Sect. VII, p. 688 *et seq.*), *the peculiar distribution of those clumps, and the qualifying influence thus brought to bear upon the effects of dirtiness*, is another matter that must be examined before proceeding further. Such distribution of the disease is frequently determined in a seemingly capricious manner, and apparently quite independently of the influence of dirtiness of houses containing infants, or of other recognisable factors. And these eccentricities of distribution of infective foci, it should again be noted, are regarded as peculiarly characteristic of the behaviour of an infectious disease. The intrinsic factors which determine the above distribution, primarily due to irregular distribution of infectious material or persons, will be discussed

in another place (sec pp. 695 and 737), but their qualifying influence upon the effects of dirtiness and the presence of infants in each district may be studied in Table XXIV.

TABLE XXIV. *An analysis of High Incidence and Low Incidence Sections; as regards distribution of attacks and of infants, size of family, and degree of dirtiness.*

	Percentage of houses attacked	Percentage of houses containing infants (under 2)	Degree of dirtiness	Size of family	Percent. of houses attacked in		Degree of dirtiness in		Number of houses included
					Houses containing infants (under 2)	Houses not containing infants (under 2)	Attacked houses	Unattacked houses	
Triangular Area :									
High Incidence Yards	70	44	56	3·3	92	65	56	55	89
Evenly affected Yards	50	29	49	—	—	—	50	48	24
Low Incidence Yards	15	28	57	3·3	21	12	65	56	66
Quadrilateral Area :									
High Incidence Sections	78	26	49	2·8	96	72	49	47	104
Low Incidence Sections	16	24	43	2·1	34	10	49	42	105

TABLE XXV. *Showing the increased incidence (percent.) of the disease upon all members of families situated within High Incidence Sections, compared with that found in Low Incidence Sections. The two areas are combined. Attacked houses only are included.*

	Fathers & Mothers	Children over 2	All persons
High Incidence Sections	33	28	35
Low Incidence Sections	26	21	28

N.B.—As many as 24 households are included in the Low Incidence Sections: their dirtiness was above the average of attacked houses.

To save needless multiplication of tables, a table showing the distribution of the attacked houses of the triangle in “yards-in-common” has been pressed into service here, since it so happens that the group of 20 high incidence yards includes practically all the clumps of attacked houses in the area, the low incidence yards representing the intervening rows of practically unattacked houses. Table XXIV, for the quadrilateral, has however been specially constructed for the present purpose, and allows

a comparison to be made between the groups of high incidence sections—specially selected to embrace all the most highly attacked clumps of houses—and the groups of sections of lowest incidence.

From Table XXIV and others preceding, it is evident in both districts, firstly, that, although the distribution of diarrhoea was decided to some extent by the proportion of houses containing infants and to a less extent by dirtiness, the above-mentioned intrinsic factors, among which the influence of yards-in-common played a very important part in the triangle, were still more potent, and numerous irregularities in correspondence of the three factors above mentioned can be made out from a detailed study of the tables.

In the second place, it is shown that the differential effect of dirtiness was almost completely smothered inside the clumps; the dirtiness of attacked houses being no greater than that of unattacked. Outside the clumps, however, in the rows of low incidence, there was the usual greater incidence displayed upon dirty houses.

Thirdly, from Table XXV, it is evident that, within these high incidence clumps or sections, the incidence upon all members of the attacked families was much greater than upon members of households situated in low incidence sections.

In Table XXVI a few sections are collected in which irregularities in diarrhoea incidence, with regard to dirt, are very marked. The added details as to the presence of infants, as to the size of the

TABLE XXVI. Sections of the districts presenting marked exceptions to the general rule that greater diarrhoea incidence is found in dirtier neighbourhoods, and less in cleaner neighbourhoods.

Dirty sections with <i>little</i> diarrhoea					Clean sections with <i>much</i> diarrhoea				
Districts	Sections	Percentage dirtiness	Percentage of houses attacked	Percentage of houses containing infants (under 2)	Sections	Percentage dirtiness	Percentage of houses attacked	Percentage of houses containing infants (under 2)	
Triangular Area	Houses 31— 35	72	0	0	Houses 155—162	33	50	12	
	„ 136—139	90	25	50	„ 178—184	31	71	57	
	„ 85—101	60	38	47	„ 11— 18	48	75	25	
	„ 64— 66	70	0	33					
Quadrilateral Area	Houses 59— 78	57	30	50	Houses 87— 93	46	85	55	
					„ 45— 58	44	64	28	
Average size of family = 3·7 children					Average size of family = 2·6 children				

families and their possible lack of immunity, and as to the sharing of common yards, show that generally none of these factors were sufficient to explain those occurrences.

The much smaller difference in dirtiness of attacked and unattacked houses in the triangle than in the quadrilateral, noted above, probably largely depends upon the different arrangement of the houses in the two areas. Thus, while in the quadrilateral the rows of houses line parallel streets and are further apart and the district is larger and more drawn out, in the triangle the district is, so to speak, folded round upon itself into as compact a mass of streets and houses as possible, the major part of the houses being much more closely placed than in the other district. Thus dirty and clean rows of houses, which are found sprinkled in alternate groups over the whole district, are brought into very close contact, with consequent easier passage of infection from one to the other, while the distinctions due to differences in cleanliness are to a great extent lost; in contrast to the arrangement in the quadrilateral, where the segregation of clean and dirty rows towards different ends of the district, out of reach of each other's qualifying influence, is so well fitted to present the greatest contrasts of the kind under notice. Again, in the triangle the capricious distribution of infection, largely influenced by the presence of yards-in-common, has occurred to a very marked degree (cf. the examples in Table XXVI), and several negative results, as regards the influence of dirt, size of family, etc., are to be wholly attributed to this fact (cf. Table XXVII).

(g) *The amount of correlation between the respective proportions of diarrhoea, of infants, and of dirtiness, throughout the various parts of the districts.* The difficult problem of the disentangling of the respective influences upon diarrhoea prevalence, of dirtiness and of houses containing infants, may be satisfactorily accomplished by working out the amount of correlation between the three separate factors from the data of the individual houses (cf. Table XXVII *b*, App.)—correlating the positive or negative history of attack of each house with its index figure of dirt, and with the number of infants (under 2) it contains. Again, the correlation of diarrhoea and dirtiness with a fourth quantity—the size of the family, was obtained; in this case the number of children per house was used, the two parents being practically constant for all houses¹.

¹ I am indebted for some valuable criticisms and suggestions upon these matters to the kindness of Mr David Heron, Galton Eugenics Laboratory, London, and Dr C. Gibson, Municipal Offices, Liverpool.

TABLE XXVII *a*. *The Coefficients of Correlation amongst the individual houses of the districts, between two sets of factors, including for each house (1) the positive or negative history of attack, (2) the number of infants (under 2), (3) the individual index figure of dirtiness, (4) the size of the family (number of children). Cf. also Table XXI.*

	Infants and dirtiness	Infants and diarrhoea	Diarrhoea and dirtiness	Diarrhoea and dirtiness (pure)
Triangular Area	0.22	0.31	0.02	-0.04
Quadrilateral Area	0.21	0.22	0.13	0.09
Both Areas	0.215	0.276	0.083	0.025
	Size of family and dirtiness	Size of family and diarrhoea	Diarrhoea and dirtiness	Diarrhoea and dirtiness (pure)
Triangular Area	0.35	-0.01	0.02	0.02
Quadrilateral Area	0.20	0.17	0.13	0.10
Both Areas	0.307	0.062	0.083	0.067
Houses not containing infants (under 2):				
Triangular Area	0.37	-0.09	0.01	0.05
Quadrilateral Area	0.16	0.18	0.17	0.14
Both Areas	0.287	0.057	0.088	0.078

Table XXVII *a* presents the two sets of coefficients of correlation thus obtained: in the fourth column is shown the pure correlation between dirt and diarrhoea, *i.e.* after the correlation values which depend wholly upon the mutual correlations of infants and diarrhoea in the first set, and of size of family and diarrhoea in the second set, have been eliminated. The results are instructive, although in the so-called pure correlation value in each set the influence of the fourth factor, and probably of other unestimated factors as well, has not been eliminated. The table shows that in the combined areas, as also in the quadrilateral, the pure correlation of diarrhoea and dirt was definite in amount in both sets. In the triangle, the pure correlation figure had a minus value, both, however, for size of family and diarrhoea, as well as for diarrhoea and dirt. But we are already prepared for this by having noted the great predominance in this area of that capricious factor discussed in Sect. V, 1 (*f*), which has determined marked irregularity in the distribution of infection throughout this district (cf. p. 649). And it will be shown that in attacked houses there was still a greater incidence within dirty houses. Moreover, it may almost certainly be taken for granted that in normal circumstances there would be a positive correlation between size of the family and diarrhoea, from the greater chance of one out of a large family being attacked, and also from the greater chance therefore of one of a large family being closely associated

with a case of diarrhoea: and therefore the minus correlation obtained points to the conclusion that the conditions were quite abnormal. In houses without infants, however, the pure correlation figure for diarrhoea and dirt is much greater, the antagonistic influence of houses containing infants having been eliminated; and this holds for both the triangle and quadrilateral. Finally, whatever the nature of the results in the triangle may be, they cannot nullify the large correlation figures obtained between diarrhoea and dirtiness in the quadrilateral area.

This is perhaps the most appropriate place for the discussion of *the mass action of houses containing infants*. It will have been already noticed that the two areas vary to some extent in the degree with which they exhibit the influences of the various causative factors. Thus, the striking factor in determining the distribution in the quadrilateral is dirtiness, babies being relatively unimportant. The converse of this holds good in the triangle. When, then, the areas were divided up into two classes of sections, containing respectively a high percentage of houses with infants (under 2) and a low percentage, on a similar principle to the division into the high incidence and low incidence sections, it was found that such houses in the high percentage sections of the quadrilateral were only slightly more affected, but in the triangle they were much more frequently attacked than such houses in the low percentage sections, the proportionate incidence of diarrhoea in the combined areas upon the two classes being as 77:56. In other words, owing to the mass action of households containing babies, the chance of an infant being attacked with diarrhoea is greatly increased by the fact of its home being closely adjacent to a number of other houses containing infants (cf. also p. 753). Owing to the greater affection of clean houses of this class, no qualification need be made for dirtiness. The high percentage sections were however located in somewhat dirtier neighbourhoods. The different distributions of breast-fed babies account for the differences above noted between the two areas (cf. Table XIX *b*).

(*h*) *The effect of dirtiness in increasing the spread of diarrhoea within the household, amongst the various members of the family.*

From Table XXVIII it is gathered that there is a decidedly greater incidence upon members of the family within dirty households than within clean. This was constantly found in all analyses. The apparently smaller excessive incidence within the dirty houses containing infants than within those not containing infants is evidently related to the peculiarly opposite incidence of the disease upon clean houses of this class. It is not improbable (although there is some doubt as to how these tables should

be read) that there is really a greater difference in houses containing infants than in others. This is supported by the fact that there was, notwithstanding the opposite tendency just mentioned, actually a much greater incidence within dirty houses, especially in the triangle—the most irregular area—when only attacked houses containing infants were considered (cf. Table XXVIII *b*). It has already been shown that the presence of babies in a house increases the liability of other members to attack (Table V), but particularly of the parents. Finally, too much must not be expected from comparisons of this kind; as owing to the marked community of infection, exhibited in the clumps, and the fact that most attacked houses of both classes are included in such clumps, it is probable that the chances of infection upon the various members of attacked households are much more equal than would at first appear,

TABLE XXVIII *a*. *The effect of dirtiness in increasing the incidence of diarrhoea within the household. The figures denote percentage incidences of attacks upon all persons in certain age-groups in the two districts.*

Both Tables are corrected for the disproportionate incidence and different proportions of individuals present at ages of greater or less susceptibility: comparison thus can only be legitimately made in one direction, *i.e.* vertically, the bottom rows of figures having no absolute, but only relative value. Houses in which dirtiness was not registered are not included.

	Houses containing infants (under 2)		Houses not containing infants (under 2)		All Houses	
	"Parents"	All persons over 2 yrs.	"Parents"	All persons over 2 yrs.	"Parents"	All persons over 2 yrs.
All Clean Houses, having dirt indices 1 or 2	15	16	17	14	16	14
All Dirty Houses, having dirt indices 3, 4 or 5	17	17	18	19	18	18

Comparison to be made in a vertical direction only.

TABLE XXVIII *b*. *A comparison between the incidence upon other members within Clean and Dirty Households, amongst attacked houses containing infants (under 2) only. A number of houses are included here in which dirtiness was not marked till after the season. No difference however results from this.*

	Triangular Area		Quadrilateral Area		* Both Areas	
	"Parents"	All persons over 2 yrs.	"Parents"	All persons over 2 yrs.	"Parents"	All persons over 2 yrs.
Clean Houses, having dirt indices 1 or 2.	15	17	25	30	20	23
Dirty Houses, having dirt indices 3, 4 or 5	24	25	38	28	28	26

Comparison to be made in a vertical direction only.

whether they do or do not contain infants, and with very little reference to the degree of dirtiness. On the other hand, that the greater incidence of dirtier houses is not due merely to association with houses containing infants is evident in Table XXV, where away from the clumps the contrast became much more marked. As regards the incidence in respect to the ages of the parents: in all houses, and in houses containing infants, the parents averaged only about three years older in the dirty houses.

(i) *Conclusions as to influence of cleanliness or dirtiness of the household upon diarrhoea incidence.*

(1) *The rather small differences in excess of dirtiness of attacked over unattacked houses*, observed in Table XVIII, and elsewhere, may be attributed to:

(a) The irregular and opposite relation to dirtiness of houses containing infants, to a great extent masking the differences exhibited amongst houses not containing infants, when all houses are added together (see p. 644 *et seq.*).

(b) The smothering of the differential influence of dirtiness in the houses of abnormally high incidence contained within the clumps; as also in the houses of abnormally low incidence situated in the intervening unattacked rows (see p. 646 *et seq.*). Since about 85 % of all attacked houses are situated in these clumps, it is quite to be expected that the differences between the aggregate dirtiness of attacked and unattacked houses, selected in an individual manner, should be exceedingly small; and we must turn rather to the distribution of these clumps, and to a study of the mass action of dirtiness, for a really good demonstration of the differential influence of the latter (cf. also Table XX).

(c) The very small actual range of dirtiness found in any one neighbourhood: it was mostly, though not invariably, confined, in any one row of houses, to two or three dirt index figures only. Thus in neighbourhoods of only moderate dirtiness, the indices might vary mostly between 2 and 3, or 2, 3 and 4—which gives a range of only from 40 to 60, or a little more. Thus a difference in dirtiness of 10 may be regarded as considerable.

(2) *On the other hand, no doubt can be entertained as to there being an important relationship between dirtiness of the household and diarrhoea incidence*; whatever the interpretation of that relationship may be. The combined facts of Tables XVIII, XIX, XX, etc., and of the associated text; along with the internal evidence of the effectiveness

of the method employed in gauging and collecting data as to dirtiness, and its proved reliability, where such could be tested by general observations, as in the correspondence of dirtiness with the number and ages of the members of a family (cf. p. 642); yield a very formidable array of evidence. The various irregularities met with have, it is hoped, been so far explained as not to appreciably qualify the large mass of positive evidence provided. Moreover, several of the tables are sufficiently convincing of themselves. General impressions are also not without a definite value of their own: thus the writer felt firmly convinced that the distribution of infection throughout the quadrilateral area was above all things determined by the varying degrees of dirtiness in different neighbourhoods. Chart VII, App., shows that the cleanest parts, in σ and τ Streets, were the earliest and at first the most heavily attacked. Infection, which appeared to be of a particularly virulent strain, remained however strictly localized, and tended to disappear early in the season. Towards π Street however, where very dirty and lax habits prevailed, infection, only at first of a moderate degree, continued to hang about, spreading later in a remarkably wholesale manner. The absence in this area of differences in social factors of other kinds has already been commented upon (cf. p. 631).

(3) *Dirtiness probably exerts no more than a moderately powerful influence*: it also appears to be a predisposing influence *of a general kind*: its manifestations are also *subject to the interacting and qualifying effects of other factors*, such as presence of babies, capricious distribution of infection, presence of much susceptible material, etc.; of which the first two at least generally wield a more important influence upon diarrhoea prevalence (cf. p. 648). In accordance with the general nature of its influence and the associated qualifying factors, the effects of cleanliness or of dirtiness are best exhibited as a *mass action*; evidenced as an exaggerated incidence upon houses lying well within dirty areas, and an exaggerated immunity upon houses situated well within clean areas (cf. p. 640); and the practical implication of which is that houses situated within dirty areas, whose unexceptionable cleanliness would elsewhere preserve them from attack, are, so to speak, drawn helplessly into the vortex of infection. Proof of this is contained in Table XX *b*, where of the houses with the low indices of 1 and 2, the 94 houses in the "clean sections" had only 39% attacked, while the 46 in "dirty sections" had 54% attacked: of more practical importance however is the contrast of the respective percentages where houses without infants

are taken alone ; this was still more striking, the percentages being as 24 : 48.

It is worth noting that the influence of this mass action is exhibited twice over in the tables ; thus :

(a) In Table XX b, any differences in the number attacked from the number of unattacked houses were almost confined to the selected "clean sections," clean and dirty houses being apparently equally attacked in the "dirty sections."

(b) The same thing was noted in Table XXIV, with regard to the "low incidence" and "high incidence" sections, respectively.

In (a) the explanation is fundamentally the same as that of (b). In the former the mass action of dirtiness has induced the presence of a much greater number of clumps ; and (b) expresses the fact that in these clumps the differential influence of dirt is completely masked or smothered.

(4) *The manner in which dirtiness of the household probably exerts its influence upon diarrhoea.* In the three sets of tables just mentioned, the further important fact is contained, that, where differences in dirtiness of attacked and unattacked houses are observable, as in the "clean sections" or in the total of "all sections," *those differences are confined to the clean end of the table.* There appears to be thus practically no recognizable differential influence exhibited by the higher degrees of dirtiness above 3, *i.e.* between the indices 3, 4, and 5. From which it may be deduced that the effect of dirtiness is not due to the presence of dirt or dust *per se*, acting for example as the vehicle of an infective organism derived from a ground or personal source, but rather to indirect influences of a general kind, as those exerted by dirtiness of general living in increasing the chances of infection : so that it is *dirtiness* and *not dirt* which is the important matter : and so that, in accordance with the above noted fact, its influence is most effectively displayed in the marked effect of *scrupulous cleanliness* in obstructing the passage of the disease, and in warding off attack ; while dirty living on the other hand acts simply by allowing the disease unrestrained freedom of spread ; dirtiness beyond a certain degree not necessarily further helping the already free course of infection. As regards infection by dirt *per se*, such a possibility is probably at least limited to dirt or dust inoculated with the specific virus ; the latter appearing, from the peculiar disposal of cases, to have only a limited and very special distribution, and not to be found in all samples of dirt. Otherwise the correlation of diarrhoea incidence to amount of dirt should

be absolute. From the above, it also follows that the influence of dirtiness in any neighbourhood is probably wholly contingent upon the local presence of infection; a high degree of dirtiness may occur without diarrhoea, where no infection has chanced to be deposited. The evidence of the charts supports this.

If dirtiness in the ways of living is then the really essential point, the question is at once suggested, Dirtiness in what particular respect?

Carelessness as regards specific faecal pollution. Here, the conclusions of the preceding sub-section (cf. Tables XXVIII *a* and *b*), as to the confirmed effects of dirtiness within the household, where babies are present, in spite of the perverse tendency to greater attack noted in the cleaner households of this class, is highly suggestive; attention being at once directed to the constant faecal pollution of the floors and atmosphere of living rooms, and to the frequent exposure of soiled napkins where they are concerned, as discussed later on (p. 660). On the other hand, amongst houses not containing infants under two years, dirtiness led to greater incidence upon the houses as a whole, as well as amongst their inmates. Here, however, it is well to remember the great community of infection exhibited amongst the attacked households of a clump, and the excessive incidence in dirty houses not containing infants may be largely determined by the presence of one or two infants in most of the clumps. Again, the presence of infants is not altogether necessary, as pollution of floors will repeatedly occur with children slightly older, when subject to an attack, and pollution of beds is not at all infrequent up to 14 years of age. Want of attention to the cleanliness of the w.c. must also be of importance, particularly where this is situated inside the house. Thus the question of dirtiness might be held to be wholly a question of carelessness in avoiding contamination from, and in removing the traces of, specific faecal pollution; and there are many good reasons for favouring this view of the case; as direct personal infection might thus be produced; or infection might be conveyed directly, or through fly-carriage, to food. With regard to the latter, the influence of dirtiness in the household in attracting, and in increasing the pertinacity of flies may prove to be of some importance.

Carelessness as regards exposure of food. Great dirtiness of the household generally went hand in hand with a tendency to leave the whole of the current food supply exposed and uncovered upon the table, at least during the whole daytime, although all the houses contained more or less suitable pantries. Perhaps the milk was less

often left exposed than the other food; the necessity for boiling it, and for protecting it to some extent, being a generally favoured precept, although no cover specially suited to the latter purpose was in use amongst the householders. It has already been remarked that, in collecting the data, special note was taken of this habit of leaving the food about. In probably a large part of the houses indexed as 4 and 5, laxity of this kind existed to a marked degree. Direct contamination of dishes and utensils used for the preparation of food might of course occur in a dirty home from the careless washing and disposal of soiled napkins.

In concluding this section, it must be regretted that there has only been room to set out the leading facts and conclusions upon this subject, and a few scanty tables. As some warrant, however, for the completeness and reliability of the latter, it may be stated that they represent the results of many months of extensive and laborious analyses. Finally, it is hoped that this enquiry will be found to have demonstrated a method of satisfactorily gauging the influence of dirt by means of actual measurements, and in establishing its importance as a factor to be seriously reckoned with in practical hygiene.

2. *Yard paving and drainage.*

In both areas the yard paving was in good repair and was of asphalt, consisting of a wide strip passing all round the backs of the houses, and also along any passage-ways leading from the street front to the back yards. This was the minimum amount of paving throughout. Where the yards were in common the strip was continuous along the back premises. On both sides of γ Street in the triangle the entire yards were asphalted, in spite of which fact, it may be noted, the incidence on the East side was about the heaviest in the area. Off the asphalt there was generally in both districts a moderate depth of unpaved land, almost without exception occupied by a garden. The yards were free from accumulations of refuse and there was a sufficient supply of ashpits or dust-bins which were emptied at regular intervals. Although an excellent sanitary procedure, in general, it is interesting to note two ways in which an impervious paving might conceivably encourage diarrhoea prevalence (cf. p. 692). Firstly, amongst dirty and careless people, with their peculiar ability to turn the best sanitary provisions into actual sources of danger, its very imperviousness makes the asphalt a means of holding and preserving infectious faecal matter upon its

surface, allowing it to dry, and be scattered as dust. Secondly, apart from the above-noted evidence as to undiminished prevalence, there was an impression received that on a sunny day the warm corners of such yards attracted and became a favourite rallying ground of flies.

3. *Sanitary conveniences and disposal of refuse.*

In the quadrilateral every house was supplied with its own water-closet and round dust receptacle of galvanised iron with removable lid. In the triangle every house had a separate closet, and the same arrangement as that just mentioned existed in the new streets referred to above. In the old streets the old privy-and-ashpit had been in use. The latter had been converted however in most cases, into water-closet or pan (pail) closet, and dry brick ashpit. The exceptions are worth noting and are indicated on the charts. There were three privy-pits still used at Nos. 6 to 10 on the South side of α Street, two at 149 to 152 in κ Street, three at the bottom of the gardens of the seven houses from 55 onwards in β Street, and two at 125 to 127 on the South side of δ Street. Conversion into pan closet and brick ashpit had occurred on the East side of β Street in Nos. 16, 17 and 18, and in 48 and 49 just opposite, also in houses 23 to 30; and again in κ Street from 140 to 145. Scavenging of refuse receptacles and closets was satisfactorily performed.

On proceeding to compare the conditions in the two areas, the remarkable fact is at once apparent that the absence of pails and privies and an exclusive provision of water-closets did not by any means preclude the occurrence of diarrhoea; for in the quadrilateral district where this arrangement exclusively obtained there was an even slightly greater personal incidence of the disease than in the triangle.

In the triangle, the heaviest incidence occurred in two adjacent groups of nine houses on the South corner of α and β Streets. These were ranged round three privy-pits, and the inmates of the five houses using these privies were attacked a little earlier than those of the other four houses which were supplied with w.c's. Owing to defective divisional railing there was free intercourse between the children of the two yards. The writer's first observations were made here; and the cluster of cases occurring around these privies, combined with what I saw of the children raking over the contents through defective doors by which the privy contents and ashes were removed, and of the diarrhoeal motions largely exposed within, made a very powerful

impression upon me that these privies were probably acting as a focus from which infection was perhaps spreading through a considerable area. It will be seen from the charts however that when my observations had been extended so as to embrace the whole triangular district, these impressions had to be considerably modified; for in several other parts cases had been occurring just as early in the season as the first in the above-mentioned focus, and it appeared also to be a matter of indifference whether the houses had water-closets or were otherwise provided.

The next most heavily affected row of houses in the triangle was that along the East side of γ Street; these houses were all provided with water-closets, but the back yards were very narrow, and owing to this the kitchens of the southern half of the row were brought within a few feet of the privy-pits, three in number, belonging to the houses facing β Street. The dividing fence was high, but flies surmounting this, or penetrating through the crevices, might easily succeed in bringing infection. On the other hand cases appeared in three of these houses, in one instance a month before any were said to have occurred in the houses to which the adjacent privies belonged. Exactly the same occurrence was also noted in the following season of 1909; and the flies about the privies on this occasion were swollen with liquid ingesta, but those of the same species inside the attacked houses had shrunken abdomens—a point which may have some significance with regard to the question of the movements of flies. In the northern half of the street the incidence was equally great, though presumably well out of the immediate sphere of influence of any privies whatever. In μ Street and other parts of the district a high prevalence was noted, away from the influence of the privies.

In the quadrilateral there were none but water-closets: there was notwithstanding, a slightly greater prevalence. Just outside the North West corner of the area there was a privy-pit attached to three old stone houses (cf. Chart II) which had occupied the site many years before the adjacent area was laid out for building purposes. It might be suggested that the greater prevalence in that corner of the district was owing to the vicinity of this privy-pit. These three houses were however visited as regularly as the other portions of the district and a special effort made to elicit a history of diarrhoea, but a negative answer, which I believe was to be thoroughly relied upon, was always obtained, although these houses contained many susceptible persons, including two grandparents, eight children—one ten months old, two sixteen months old, and one

two years old. Incidentally the fact that the virulent outbreak at that end of π Street did not succeed in crossing the intervening 30 or 40 yards of open grass land to these susceptible children is also of peculiar interest. Moreover, I felt quite certain that these old cottagers had no communication with the collier population at the end of π Street. No other privies or pail closets existed within several hundred yards of the centre of the district.

With regard to the much debated question of water-carriage versus conservancy systems of disposal of excreta, the subject of household dirtiness again presents itself. As regards any differences observed with respect to the spread of diarrhoea or enteric fever, it is not improbable that the crux of the whole matter lies in the *cleanly working* of the water-closet. In the dirtiest districts, blocking of the outlet, with practical conversion of many of the w.c's of a district into so many objectionable pail-closets, neglect of flushing, and unnecessary soiling of the basin and of other parts of the structure, are of very frequent occurrence. Again, the *film of faecal matter* that is so commonly found adhering to the basin in dirty houses, may possibly present as great an area of infection as the conservancy pan itself to a fly carrier. With regard to the latter, however, the cooler atmosphere of the water-closet appears to be distinctly repellent to the fly. Moreover, the exposure of diarrhoeal motions in a confined space frequented by all the members of the family in turn must be regarded as a very mischievous feature of the conservancy system (cf. Bruce Low, 1887-8, p. 127 *et seq.*).

Faecal pollution within the household. On the other hand, certain facts will now be mentioned, which are quite outside the question of water-closet versus conservancy pan, but which are probably much more closely connected with the question of faecal infection in diarrhoea. Whatever arrangements may obtain as to the foregoing, there still persist, in most houses of the class met with in the two districts, the same peculiarly lax methods of dealing with the excreta of young children up to the age of three or four years; methods which although unwittingly allowed, from long custom, and though more difficult to remedy, yet deserve to attract the same evil notoriety as that of the old cesspool in the basement. I found that children of this age generally passed their motions within the house, in the presence of other members of the family, and frequently in the room used as kitchen and eating room, or in the scullery which communicated directly with the latter and often led also directly through into the pantry (see Chart III, App.). In the case of infants, the napkins were changed in the same rooms and

put to soak in the scullery, not always being completely covered with water, and no disinfectants being as a rule applied to the vessels in which they were washed. When napkins were discontinued the commode chair was used, being usually kept in one of the same two rooms; underneath the chair there was placed a tin receptacle, or a chamber brought only when required from the bedroom. The receptacles were emptied into the w.c. and rinsed with water from the scullery tap where the dishes were also washed. No disinfectants were used afterwards. In a few more careful households however the chair was placed in the w.c. and the child sent there to use it.

In non-diarrhoeal diseases with infective motions, such methods of dealing with the excreta of children within the living rooms and in the presence of the other members of the family would be considered highly dangerous; but when the symptom of diarrhoea is a marked accompaniment of the disease the conditions are many times worse, owing to the loss of voluntary control of defaecation which is so common a feature of the disease in children. Thus the receptacle which had to be brought from the bedroom as often as not was brought too late to prevent the soiling of the floor, the child using the chair without it. Moreover, in bad cases the loss of control was often so great that the diarrhoeal motions would run from the child upon the floor or pavement as it walked about. Soiling of the bed at night was noted as quite common in children up to ten years of age and even above, and it was not unusual to find the whole available bedding of the household in the wash when an outbreak had "gone through" the house. Even with careful mothers, where a baby and one or two young children were affected, gross pollution of the floors and yard paving was often witnessed, the mothers being unable to pay the amount of attention necessary to ensure against such occurrences. It must further be noted that young children old enough to walk are seldom invalided, but are generally able to go about throughout their attack.

In view of all these facts, it is evident, here perhaps even more so than with regard to the successful working of the water-closet, that household cleanliness is a matter of supreme importance; otherwise every part of an attacked household must speedily become saturated with diarrhoea infection. To convey a just idea of the important part undoubtedly played by filthy habits, it may be mentioned that in a number of houses in the dirtiest corners of both districts it was found that young children, and not only those of infant years, were in the habit of frequently depositing their faeces upon the pavement around

the house, apparently with the full knowledge of their parents. Next door to such a house in π Street, where most of the children had been attacked, I had the opportunity in 1909 of investigating an outbreak, related in point of time to the latter, in a highly intelligent and scrupulously clean family of advanced adult age, who courted a most minute and lengthy enquiry into every relevant detail: association with neighbours, and carelessness with regard to food, could be absolutely excluded. There remained but this one fact: habitual pollution of the pavement of the adjoining house with diarrhoeal motions: the inference as to infection from that source—probably by some carrier agency—could thus be hardly avoided.

To this might be added one more of those actual personal experiences which are altogether unsurpassed for the lasting impression they leave upon the mind of the enquirer. It illustrates the need for readjustment of perspective regarding the relative importance of causes of diarrhoea spread. The investigation of a series of cases was begun by making the usual copious notes as to closet accommodation, drainage, etc. But the inconsequence of the enquiries upon these external matters was only too plainly apparent when I entered the house and saw: A dirty, untidy room: the current food supply exposed upon the table: the mother distracted with an armful of young children,—none of whom could have been old enough to use the w.c.: *and a recent diarrhoeal motion lying unheeded on the floor!*

Conclusions. As regards diarrhoea, then, the question of disposal of the excreta is apparently not met, to any extent, by the conversion of privy and pan closets into w.c.'s.

Considering the wholesale faecal contamination inside the house, mostly by young children who cannot use the conveniences provided for adults, the little extra risk from the exposure of an infected surface at some distance outside the house may not perhaps after all make so much difference.

With regard to diphtheria, Davies found at Bristol that the epidemic was specially located in the outlying newly-built districts, which were specially favoured as residences by the healthy and prosperous young families of the working-class population. The inference being that one might almost traverse a town and be able to point out those parts where diphtheria is most likely to become epidemic. The explanation of this very useful observation is more simple than at first appears: it probably lies in the fact that it is in the houses of this class that young children of susceptible age are most thickly collected. And

with regard to diarrhoea also, it was in this same class of house that I found the disease most prevalent; infants and young children being there more common than in the old confined and insanitary dwellings in the centre of the town. I was surprised at not being able to discover in the latter situation, which might be called the slum part of the town, a prevalence as great as that in the two large areas. But the above new property generally includes houses exclusively provided with water-closets and built on the most modern sanitary principles: it thus comes about that the water-closet districts or, again, water-closet towns as a whole, may show, other things being equal, a greater prevalence of diarrhoea than privy and pan-closet districts or towns respectively: of which fact, moreover, there is abundant statistical evidence. Thus, of the 14 largest English towns, Liverpool, which has for some years been exclusively provided with water-closets—at least as far back as 1899—had the highest diarrhoea mortality rate for the ten years 1897–1906; although the 14 large towns included several with an actual preponderance of midden-privies or of pail closets over the number of water-closets¹. Here it may be that with a large slum population the proportion of deaths to cases may be unusually high or that dirty habits of living have persisted to such an extent as to render the institution of w.c.'s little improvement upon the old privies. Again, the example of rural districts, upon which as well as upon city conditions the writer can speak from official experience, may be quoted, with their exceedingly low diarrhoea mortality rates, in spite of the intimate and appalling admixture of gaping privy pits with the small confined dwellings of their villages. This must, again, be largely attributed to the smaller density of the infant population, resulting in part from the lower birth-rate, along with the greater cleanliness, particularly as regards care with food, of the agricultural population. With regard to the other side of the question and in relation to enteric fever, Boobyer (1908) has published for a number of years comparisons as to the relative incidence

¹ A very pointed illustration of this argument has just come under the writer's notice in the current number of the *Lancet* (Sept. 10, 1910, p. 852) with regard to Rhondda Urban District, which is a colliery area with a very high diarrhoea rate. The exceptionally high infantile mortality rate of 190 in the 10 years preceding 1909 is remarked upon: it was 138 for England and Wales. Yet “*for many years past the prevailing system in the Rhondda has been that of water-carriage, and among the 25,000 houses there are only 200 which are not provided with water-closets.*” That is to say, notwithstanding the introduction of the system of water-carriage the principal cause of the mortality still remains unrevealed. The question may be asked, Do not the dirty habits of living noted above, particularly amongst colliers, with regard to faecal pollution of the household and carelessness with food and in the management of the w.c., constitute this cause?

at Nottingham upon midden privies, pail-closets, waste-water closets and water-closets. The respective incidences were roughly 21 : 7 : 4 : 2 during the four years, 1905-8.

Comparisons between towns as to the effects of the various systems of disposal of excreta upon diarrhoea prevalence cannot therefore give reliable results, while the more important factors of density of the infant population, of dirtiness and of divergent case mortalities are left out of account; apart from the very minor part the former may really play in faecal infection. But whatever may have been deduced hitherto from comparisons of questionable value, it is impossible to avoid the conclusion, on general principles, that the water-carriage system contributes, *or might be made to do so*, a distinct and necessary sanitary reform.

4. *Stables and Manure Pits. Fly Nuisance.*

The importance of stables depends upon the accompanying accumulations of horse-manure, which furnish the commonest breeding grounds for flies, and upon the reputed association of diarrhoea and the *B. enteritidis* sporogenes with which horse-manure abounds. No systematic fly-counts were made during the season, but many observations were made as to the relative abundance of flies at different times and as to the presence of collections of refuse, stables and manure heaps, from which the fly-swarms of the various districts probably emanated. The manure heaps and stables are marked on Charts I, II and III, App.

In the triangle (Chart I, App.), there was only one manure heap in which the larvae and pupae of the house-fly were discovered. It was at the back of a baker's shop, right against the side fence of No. 84 in γ Street; it swarmed with larvae; fowls however were continually on top feeding upon the latter, and the manure was frequently moved; possibly however a large number of flies were occasionally hatched, as the residents in the houses adjoining complained a good deal of flies, and it will be seen that the two rows adjacent had a very heavy incidence of diarrhoea. They included however some of the dirtiest houses in the district, with a high percentage of infants. No flies appeared to be hatched from the other small heaps of manure behind Nos. 27 and 144, in the stable at the back of 154, nor in the heaps outside the district opposite 142, nor in the stable just mentioned. There was no nearness of stables to account for the high incidence in α and β Streets, or in μ Street.

In the quadrilateral area (Chart II, App.), a milkman's stable was placed against the back wall of No. 205 in σ Street and a swarm of flies was found in the manure heap. The cases became thicker at the end of that street, but this was attributable to the fact that the dirtiness of the people there was very great, in marked contrast to the particularly clean and well-appointed houses of the people further along. This abrupt change in the character of the householders resulted from the fact that it was not so desirable a residential site, being a very noisy corner; there being also two shops adjacent, one of which was a fried fish shop. A stable and small manure heap were also found at the back of No. 156; no fly larvae were found on the two visits made; there was a fairly high incidence of diarrhoea in the houses adjoining, most of which had back yards in common. A large manure heap, *which may have yielded the chief supply of flies to the district*, was situated outside a stable, over the wall from the west ends of σ and τ Streets. A large number of fowls were however always upon it. It will be seen also that the parts of the district nearest had not an unusual incidence of diarrhoea. The houses at the end of π Street, which were so heavily attacked, were, again, furthest away from this heap; and indeed from all other stables, whether situated within or without the district; so that in this case there seemed to be no very special prevalence of flies to explain the unusual prevalence of diarrhoea.

On a careful review of these facts and a close study of the charts, one important fact was however demonstrated. Whatever evidence was obtained seemed to point to the fact that flies, if they were, after all, more or less necessary agents of the spread of the disease, *did not bring infection with them from the manure heaps where they had been bred*; otherwise, the incidence of diarrhoea would have been greater around the stables or at the points where the swarms of flies entered the districts; *but that they only acted as carriers when, in the course of their wanderings, they came across an infected household where they could obtain infected matter to carry*. Cf. also the evidence of the prevalence curves (Sect. VII). It would also be most reasonable to assume that their power of infection is greatest immediately around such an infective focus, the infected matter being there most thickly deposited by the fly; and the evidence adduced hereafter as to the tendency to grouping of infected houses gives support to that assumption.

The far end of π Street, alluded to as the neighbourhood of heaviest incidence, was surrounded on the north and west by open country, with no stables or refuse heaps within a good part of a mile. The houses

however showed a greater want of cleanliness than any in the district. It is possible that the greater incidence of diarrhoea upon such dirty houses depends largely upon the special influence that dirtiness exerts in attracting the attention of the flies that pass that way. The fact remains, however, that the high incidence was at least not due to mere nearness of the houses to centres where flies were produced.

This fact was particularly well illustrated in a third small area investigated, which furnished as complete a demonstration of the point as well could be desired (cf. Chart III, App.).

The supply of flies throughout this latter area must have been mainly derived from the far end of ϵ Street, where a large heap of manure, from the livery stables opposite, lay always exposed and full of fly larvae; as there were no other breeding grounds within a considerable distance; and because from this manure heap the flies could be traced all along ϵ Street in lessening numbers, settled during a cold change upon the door and window frames, the doors opposite the heap being almost black with them at such times. The diarrhoea however had its least incidence upon the houses in ϵ Street, nearest the heap, and most in θ Street, the part farthest away. It was in θ Street, and at the end of η Street adjoining, that the first foci of the disease were established, the flies thus apparently only acting as carriers from the time that they penetrated to and had established their quarters amongst these more distant houses, afterwards spreading infection outwards from this focus. In June and July scattered cases occurred; two out of the only three cases occurring in ϵ Street appearing in the latter month. During the first three weeks of August one of the typical explosive outbreaks occurred in θ and η Streets. During this time however no fresh cases occurred in ϵ Street, from which direction any fresh supplies of flies at this time must have been derived.

It was thought worth while, assuming that the rôle of flies is one of superior importance, to test what effect the aspect of a house had upon its chances of attack from diarrhoea; for the reason that the flies appeared to especially frequent the sunniest side of the house. Since the people in the two large areas, almost without exception, lived mostly in the rear part of the house, where the kitchen and pantry are also situated, it was resolved to test whether houses with their rear part facing the south and west really had more diarrhoea. The proportion affected of such houses was, however, found to be about equal to that of houses with the rear facing the north or east. In the quadrilateral, however, the former group had a slightly higher incidence. The districts are however

much too small for testing this point, so many other variables affecting the comparison. If however the rows of houses along κ Street and σ Street, whose very small incidence could be attributed to irregular prominence of causal factors elsewhere not unduly evident, could with fairness be excluded, the proportion affected in the two districts of houses with rears facing south and west would be well above that of those having the other aspect; and indeed even a casual inspection of the charts seems to give one the impression that such houses were excessively affected.

Finally, it may be stated that, with the exception of the observation as to *non-carriage of infection from the flies' breeding ground*, no conclusion of note with regard to the areas examined was arrived at, either for or against the question of fly-carriage. It was often suspected, and in the case of District III it was certain, that *neighbourhoods having a low fly prevalence might be more heavily attacked than those with a very high fly prevalence*, and vice versa. Again, the assumption that has been provisionally made, in the absence of exact knowledge as to the habits and movements of flies, that flies are to be found thickest around their focus of origin and gradually spread out from thence in ever decreasing numbers, was most convincingly upheld in the case of District III. The possibility must not however be lost sight of that it may hereafter be shown that fresh swarms of flies may be frequently blown or become transported, immediately after hatching, *en masse* to considerable distances from their breeding grounds.

The subject of fly-carriage in diarrhoea will be still further developed in two later sections of this paper (see Sects. VII 2 (c), p. 707 and VII 3 (b), p. 727).

VI. FOOD.

Under this heading all articles of *food* and *drink* are dealt with: special reference is made to the milk-supply, the water-supply and the suggested part played by fruit and other solid foods.

The belief in a causative relation between food and diarrhoea is a very old one and, from various primitive conceptions, it has passed through several interesting evolutionary stages, none of which, it is important to note, may yet be said to be completely and finally disposed of. In pre-bacteriological days diarrhoea had come to be attributed to purely physical causes, such as indigestibility of food; and the special incidence upon infants likewise to artificial feeding. But the discovery

of the bacterial basis of infective disease at once suggested the possibility of food infection. At first, no doubt from the fact that the larger micro-organisms and those producing fermentation were the first to become known, it was suggested that diarrhoea was due simply to ordinary fermentative processes occurring in the food, whether within or without the body. Johnston's conclusions (1878-9, p. 212) furnish an interesting and instructive illustration of this point. He says: "I, therefore, consider that (a) diarrhoea, as it affects both adults and infants during the summer months, owes its origin in the great majority of instances to the introduction of minute living organisms (bacteria) into the system by means of air or in food; and (b) the disease depends upon putrefactive changes in the bowel contents, which changes are correlative to the development and multiplication of these microscopical organisms."

From the context it is evident that he is here alluding to no one organism in particular, but to the ordinary fermentation-producing organisms in general, of a non-pathogenic type and such as those usually found in sewer gas. Ballard (1887-8), however, ten years later, raised diarrhoea, or rather our conception of it, to the rank of a specific infective disease, limiting the cause to the agency of one specific micro-organism. He still, however, appeared to attach most importance to the fermentative changes in the food, and to the substances so produced, both outside as well as inside the body; thus emphasising the saprophytic phase of the organism (*ibid.* p. 7). More recently, organisms of a more strictly pathogenic type have been put forward. Following up Ballard's conclusions, later observers searched for an organism leading a largely saprophytic existence and gaining admission to articles of food and drink. For obvious reasons milk in particular fell heavily under suspicion, and an important question arose as to the stage in milk production at which infection occurred. Stress was laid upon the possibility of infection at the farm and during distribution; but lately close attention has been directed to the question of infection occurring after the milk has arrived within the home; such infection being perhaps derived from a personal source. The method by which infection is introduced into the milk or food has been ascribed to general causes, such as conveyance in dust, but recently carriage of infection by flies and inoculation of food by that means has received most attention. Interest had centred, however, at a somewhat earlier date around the question of the peculiar immunity from fatal diarrhoea experienced by infants fed at the breast. Thus Hope (1904, p. 42), in investigating, during the years 1884-6, the circumstances of over 1000 deaths of infants, taken consecutively, had found

that during the season the deaths of infants under 3 months of age, amongst those fed on breast-milk, were fifteen times less than they were amongst an equal number fed wholly or partially on artificial foods. And this matter will be the first to receive special consideration.

1. *Milk.*

(a) *Human and Cow's Milk in Infant Feeding.*

In Table XXX the methods of feeding young children, in the two districts, under two years are indicated. These were practically restricted to two—breast feeding and feeding on cow's milk. After about six months of age it was a general custom to supplement either of these foods by crusts from the table, a practice which gradually opened the way for a more varied diet; so that in the second year, even though still on the breast or the bottle, most infants received liberal helpings from the table at which their parents fed.

The articles of food which proved to be of really vital importance with regard to the incidence of diarrhoea were two—*human milk* and *cow's milk*; and these presented remarkably opposite qualities in this respect. The addition to either of these dietaries of crusts or other articles from the table appeared to have no effect, at least up to the ninth month, upon the markedly low incidence of the disease that accompanied the giving of breast milk or upon the very high incidence obtaining where breast milk was replaced by cow's milk.

The percentage incidence of the disease upon the first year where breast milk was given was 32 %, and 90 % in the case of cow's milk; and the younger the child when the substitution for cow's milk took place the more liable did it appear to be to contract diarrhoea. The method of feeding had also a considerable effect upon the duration of attack and the tendency to recurrence in the same season, as shown in the following table, formed upon the data dealt with in Table XXX:

TABLE XXIX.

	Breast milk only	Breast & cow's milk, or crust	Cow's milk, etc.
Average <i>duration</i> of { Under 1 year	8	8	14
illness in days { 1 to 2 years	7	24	11
Percentage having <i>recurrent</i> attacks, under 2 years	0	8	15

This enquiry, then, furnishes an interesting demonstration, and probably one that is unique as regards data obtained in a consecutive manner and relating to diarrhoea sickness, of the fact that the above described low diarrhoeal death rate of breast-fed infants depends, not merely upon the lessened chance of an attack ending fatally, but also to a large extent upon the lessened chance of attack occurring at all.

TABLE XXX. *Showing the method of feeding of 105 children under 2 years in the triangular and quadrilateral areas, and the proportion in each feeding group affected with diarrhoea. The latter is expressed as a fraction by placing the number attacked over the total number fed in any specified way; the equivalent percentage is also in some cases placed beside the fraction.*

Method of feeding	First year	Second year	First year				Second year			
			(0—3) mths.	(3—6) mths.	(6—9) mths.	(9—12) mths.	(12—15) mths.	(15—18) mths.	(18—21) mths.	(21—24) mths.
Breast milk, wholly or partially	$\frac{15}{46}$ 32	$\frac{5}{12}$ 41	$\frac{0}{11}$ 0	$\frac{3}{11}$ 27	$\frac{4}{16}$ 26	$\frac{8}{9}$ 88	$\frac{4}{6}$ 66	$\frac{1}{2}$ 50	$\frac{0}{2}$ 0	$\frac{0}{2}$ 0
Cow's milk, wholly or partially (but no breast milk)	$\frac{18}{20}$ 90	$\frac{3}{12}$ 61	—	$\frac{6}{6}$ 100	$\frac{5}{5}$ 100	$\frac{7}{9}$ 77	$\frac{4}{8}$ 50	$\frac{5}{7}$ 71	$\frac{2}{3}$ 66	$\frac{2}{2}$ 100
Details of feeding:										
Breast milk only ...	$\frac{8}{27}$ 29	$\frac{1}{2}$ 50	$\frac{0}{9}$	$\frac{2}{8}$	$\frac{3}{7}$	$\frac{3}{3}$	$\frac{1}{1}$	$\frac{0}{1}$	—	—
Breast milk, crusts, etc. ...	$\frac{6}{16}$ 31	$\frac{2}{6}$ 33	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{1}{7}$	$\frac{4}{5}$	2	—	$\frac{0}{1}$	$\frac{0}{1}$
Breast & Cow's milk, & crusts or farinaceous foods, etc.	$\frac{2}{3}$ 66	$\frac{2}{4}$ 50	—	$\frac{1}{1}$	$\frac{0}{1}$	$\frac{1}{1}$	$\frac{1}{1}$	$\frac{1}{1}$	$\frac{0}{1}$	$\frac{0}{1}$
Cow's milk only ...	$\frac{12}{100}$ 100	$\frac{4}{44}$ 44	—	$\frac{4}{4}$	$\frac{5}{5}$	$\frac{3}{3}$	$\frac{0}{2}$	$\frac{2}{4}$	—	$\frac{2}{2}$ 100
Cow's milk & crusts or farina- aceous foods, etc.	$\frac{6}{8}$ 75	$\frac{0}{12}$ 75	—	$\frac{2}{2}$	—	$\frac{4}{6}$	$\frac{4}{6}$	$\frac{3}{3}$	$\frac{2}{3}$	—
Condensed milk only (Nestlé)	$\frac{0}{2}$ 0	—	$\frac{0}{1}$	—	$\frac{0}{1}$	—	—	—	—	—
Same food as parents ...	—	$\frac{4}{4}$ 100	—	—	—	—	$\frac{1}{1}$	$\frac{2}{2}$	$\frac{1}{1}$	—
Feeding unrecorded ...	$\frac{0}{6}$ 0	$\frac{8}{15}$ 53	$\frac{0}{1}$	—	$\frac{0}{1}$	$\frac{0}{3}$	$\frac{2}{4}$	—	$\frac{5}{5}$	$\frac{1}{1}$ 100
Totals in both districts ...	$\frac{33}{73}$ 45	$\frac{30}{52}$ 57	$\frac{0}{13}$ 0	$\frac{9}{17}$ 52	$\frac{9}{22}$ 40	$\frac{15}{31}$ 71	$\frac{11}{19}$ 57	$\frac{8}{11}$ 72	$\frac{8}{14}$ 64	$\frac{3}{3}$ 100

Details as to boiling of milk before feeding.

Cow's milk wholly or partially (no breast milk)	Boiled	$\frac{17}{18}$ 94	$\frac{10}{66}$ 66	—	$\frac{5}{5}$	$\frac{5}{5}$	$\frac{7}{8}$	$\frac{4}{6}$	$\frac{3}{4}$	$\frac{2}{3}$	$\frac{1}{2}$
	Unboiled or sometimes so	$\frac{1}{2}$ 50	$\frac{3}{6}$ 50	—	$\frac{1}{1}$	—	$\frac{0}{1}$	$\frac{0}{2}$	$\frac{2}{3}$	—	$\frac{1}{1}$
Cow's milk only	Boiled	$\frac{12}{100}$ 100	$\frac{3}{42}$ 42	—	$\frac{4}{4}$	$\frac{5}{5}$	$\frac{3}{3}$	$\frac{0}{2}$	$\frac{2}{3}$	—	$\frac{1}{2}$
	Unboiled or sometimes so	$\frac{0}{6}$ 0	$\frac{1}{2}$ 50	—	—	—	—	—	$\frac{0}{1}$	—	$\frac{1}{1}$
Cow's milk and crusts or farinaceous foods	Boiled	$\frac{5}{6}$ 83	$\frac{7}{8}$ 87	—	$\frac{1}{1}$	—	$\frac{4}{5}$	$\frac{4}{4}$	$\frac{1}{1}$	$\frac{2}{3}$	—
	Unboiled or sometimes so	$\frac{1}{2}$ 50	$\frac{2}{4}$ 50	—	$\frac{1}{1}$	—	$\frac{0}{1}$	$\frac{0}{2}$	$\frac{2}{2}$	—	—

As regards the explanation of these facts, it is conceivable that breast milk may act in one or both of two ways: either it plays a passive part, where it is partaken of to the exclusion of all other foods; thus ensuring, firstly, a wholly germ-free diet; and secondly, the exclusion of foreign—and consequently irritant and less nourishing—milks and other foods: or, its rôle is an active one, exercising, by means of certain vital properties, an inhibitive or bactericidal action upon any infective bodies introduced in the food. The cow's milk, again, may act either mechanically, by its irritant action upon digestion, being a foreign milk and to that extent an unnatural food: or specifically, by being the vehicle through which the specific virus is generally introduced into the body. Of course the cow's milk might conceivably produce its effect in both of these ways, the former one paving the way for the latter.

There were two reasons for giving serious consideration to the merely irritative effect of cow's milk upon digestion. The first was that the younger the child, and consequently the more limited and the less adaptable its digestive powers, the greater was the incidence upon those fed on that milk. Under 9 months every child so fed contracted diarrhoea. The second was, that boiling the milk before feeding had no effect in preventing the disease; thus making it difficult to at least believe that the diarrhoea was due to a living and specific infective agent. It is also possible that boiling actually increased the irritant properties of the foreign milk, just as it impairs the properties of the natural milk.

The boiling of milk. Turning to the question of boiling: the results obtained were very remarkable. Boiling appeared to produce no effect at all; or if anything, it appeared to increase the liability to diarrhoea; perhaps in the way suggested above. If however it be supposed that the cause of diarrhoea is generally introduced in milk, and is a living virus, boiling before feeding, even if the routine of feeding is not always in accord with scientifically perfect asepsis, should at least produce *some* decrease in the incidence of diarrhoea. The fact that boiling had no effect may be interpreted in one or more of three ways: either the actual cause of the disease is not affected by boiling—in that case recalling the interesting theories that it may depend simply upon the presence of toxins in milk as a direct or merely predisposing cause, or upon the presence of a sporing organism which can resist the heat: or secondly, it may point to the fact that infection by milk is not really of great importance as a method of spread, that other foods are just

as important, or that some other method must be sought, as *e.g.* that of direct personal infection: or thirdly, the process of boiling may, and probably does, destroy certain vital properties of milk which inhibit the growth of disease organisms, whether or not that protection may depend upon the presence of the inhibitory lactic acid organisms; or increases, by the chemical changes it induces, the irritant action of a foreign milk.

It will be noticed that in these districts the practice of boiling the milk, before feeding infants under one year, was almost universal; a practice which was no doubt the good result of the many years of persistent local medical teaching upon the subject. The employment of the modern tubeless feeding bottle was also, for similar reasons, quite the usual practice. Milk partaken of by children, above the age of 2 years, was usually derived from the general household supply, which in summer at least was generally boiled on receipt. In 71% of 53 houses canvassed it was recorded that the milk was "generally boiled" or "always boiled." At ages 2, 3 and 4, where a mixed diet was partaken of with the addition of drinks of milk, there was some slight indication of a greater incidence with unboiled milk; but the figures were too small and incomplete—data not being systematically gathered upon this point—to draw reliable conclusions from; there being only 11 children, at the three ages, recorded as partaking of unboiled milk.

From what has been here put forward as to the boiling of milk, the proffering of advice to the general public to boil their milk in summer would appear to be so much labour in vain, as regards the prevention of diarrhoea. It is important to note, however, that the evidence obtained as to the ineffectiveness of boiling, as a home procedure amongst an uninstructed public, is not directly antagonistic to the practice of distributing milk, produced, sterilized, and delivered in sealed bottles, from scientifically organised and controlled milk depôts; although the absence of any effect at all, in the home treatment of milk, rather suggests the inutility of boiling, under most circumstances.

The above conclusions might be supported by quotations from several recent papers on this subject (cf. some in the list quoted at the foot of this paper); and it may be added that all the possible explanations of the parts played by breast milk, cow's milk, and by boiling, suggested above, have at various times, and even up to the present, received serious support. Stawell's remarks (1899, pp. 153—7) of 10 years ago have apparently anticipated the present attitude.

Referring to the fact of *stale milk*, i.e. *milk which has lost its first freshness*, being constantly associated with a high diarrhoea incidence, whether it be taken boiled or unboiled, he says "no amount of special care will certainly prevent diarrhoea" with "ordinary bought milk," "even if that milk is pasteurised on delivery." "All bought milk is for practical purposes to be regarded as 'stale milk.'" Upon ordering the keeping of a cow, for the supply of perfectly fresh milk, he was able to bring infant patients through the summer without diarrhoea. The keeping of a goat affords a somewhat more economical means of attaining the same end.

This enquiry as to infant feeding throws a very interesting light upon *questions of age incidence*. It seems that the second year of life shows the greatest attack incidence, but it might be argued that, as in the case of mortality, the first year would exhibit the greatest susceptibility, were it not veiled by the large amount of breast feeding during that period. Again, the high incidence of the second year may be considered to be abnormally raised by the numbers of highly susceptible children who have, up to that time, been protected from attack by breast feeding. These suggestions are in fact borne out by examining the incidence in those fed otherwise than upon the breast, from the earliest months onward. In the second and following trimestra, where artificial feeding has been instituted, the incidence is greatest of all, being 100%; but from that period onwards through the first, second, third, and following years there is a gradual and continual fall.

As regards the rôle of cow's milk (cf. also p. 671), it is a remarkable fact that the rise of diarrhoea incidence to a maximum in the second year with subsequent gradual decline, corresponded exactly with the frequency with which cow's milk appeared in the dietary of children throughout the first few years of life. The most constant article of diet of any one period of childhood is probably cow's milk during the second twelve-monthly period of life. It is the staple article of diet at this time, however varied the method of feeding may be before or afterwards. It was found by special observations that most cow's milk was given at this period, but that it was also given largely, although with gradually decreasing frequency, in the years immediately succeeding this age. Children above two almost invariably partook of an ordinary mixed diet, being fed from the table along with their parents; but according to the tenderness of their years, it was generally the rule to supplement these meals with drinks of milk, as being a natural and even necessary food for children of such an age; the

practice being gradually discontinued as they grow up; and it is important to note that the incidence upon the third year was actually as great as that upon the first year of life, viz., 45 % in all houses. These somewhat striking correspondences then suggest the question as to how far the greater susceptibility of certain age-periods is due to the direct effect of cow's milk and the greater frequency with which it enters into the dietary. As the result of a special analysis it was found that the proportion of "first cases" amongst those fed on cow's milk and amongst those on the breast was about the same. Thus, setting aside domestic infection for the moment, there was no indication that the cow's milk had played a specially important part, as regards introducing diarrhoea within the household.

As regards the rôle of breast milk. In accepting the simplest hypothesis, that susceptibility to diarrhoea decreases from the earliest months upwards, it is practically implied that breast milk must exercise an active inhibitive influence upon diarrhoea infection. This influence was most marked up till the end of the ninth month, only 18 % so fed being attacked, as against 100 % of those fed on cow's milk without the breast. After that time, probably owing to the impaired quality of the breast milk, the protective influence was largely lost. Disturbances due to teething should be remembered at this period. The large proportion of crusts and scraps from the table that enter into the dietary at this time might also be held accountable for this sudden change; but that, before the end of the ninth month, the 11 children so fed showed a remarkably low incidence, even less than those said to be wholly breast fed. From the latter fact it is evident that before this period, at any rate, the notable quantities of household dirt and dust known to be introduced on these crusts appeared to convey no infection, or at least were rendered completely harmless by the admixture with breast milk. Turning to the 22 houses containing breast-fed babies under six months, only three of which babies were affected, it is found that 66 % of such houses were attacked houses. This was a proportionately high figure, since only 70 % of all houses with children under one year were attacked, and the latter include many houses where the infant under one year was the only child attacked. The fact that the breast-fed infants remained free in the presence of all this infection (in one case with as many as four other members of the family attacked), is again evidence of the positive inhibitive action of breast milk.

During the season, no cases of diarrhoea were found under three months, but in the November following a typical case was found, seven

weeks old, and fed wholly upon the breast. Even at this early age then, there is an appreciable susceptibility, and breast milk is not completely protective against attack. Further than this, five cases, or 20 % of those wholly breast-fed, under nine months, were attacked: in two of these the mother developed diarrhoea one and five days respectively before the child; but in two others no association with attacked persons could be discovered. Similar results were found amongst children partially breast-fed.

(b) *The Milk-Supply as a suggested source of infection.*

When milk became suspected as a vehicle of infection, one suggestion put forward was that infection generally occurred at some time previous to, or during, the distribution of milk to the householder; milk thus becoming an important means of *introducing* infection into the home. This view of the case which has always received a great deal of attention will now be discussed in reference to the relations of the milk supply and diarrhoea prevalence in the triangular and quadrilateral districts. The percentage of attacked households in the round of each milkman is shown in Table XXXI. There were a large number of milkmen, 21 in all, 13 in one district and 15 in the other. Milkmen *D*, *E*, *F*, *G*, *H*, *J*, and *K*, delivered milk in both districts. There should thus be ample material provided for demonstrating special infection of particular milk-rounds, should such be at all common in diarrhoea. The comparison instituted, however, yielded no evidence that the attacked households were confined to certain milk-rounds, or that some rounds were very much more affected than others.

In the triangle the four largest milk rounds, comprising from 9 to 57 customers each, showed a curiously equal incidence upon the houses included in each round, varying only from 50 % to 55 %. In the 9 smaller ones there was a constant tendency to a similarly equal incidence, and the incidence upon all the milk-rounds in the district was 53 %. Again, as regards diarrhoea incidence, the various rounds taken separately were found to vary fairly regularly together with the variations in incidence upon the whole section in which they were contained. The great affection of 9 out of 10 houses in Section I in the round of milkman *A*, might at first appear to implicate the milk-supply, but in Section IV only 1 out of 11 houses were attacked, though supplied with milk from exactly the same source. Absolutely no

variation in incidence, therefore, could be set down to the influence of the milk-supply.

In the quadrilateral area, the incidence upon the various milk-rounds appears at first to be rather uneven. It will be seen, however, by consulting the widely differing percentage incidences of diarrhoea upon the various sections of the area, placed at the bottom of each table, that this can be largely referred to the very unequal incidence of the disease upon the different ends of the district, and to the fact that some milk-rounds were mostly limited to one and some to the other end: the incidence of the disease upon the different sections of the triangle was, on the other hand, much more even. In the quadrilateral, for example, the rounds of *R* and *S* are practically limited to Sections III and IV, where the diarrhoea was absent altogether from long rows

TABLE XXXI. *Showing the proportion of houses attacked in the round of each milk seller. In the fractions the number of houses attacked are placed above the number of all houses—the equivalent percentage being also given in some cases. The districts are divided into sections to afford an opportunity of studying the local distribution.*

Triangular District.							
Milkmen or milk- rounds	Sections of the district					Whole district	Percentages of houses attacked
	I Houses (1-40)	II (41-84)	III (85-130)	IV (131-154)	V (155-184)		
A	$\frac{9}{10}$	$\frac{4}{7}$	$\frac{10}{21}$	$\frac{1}{11}$	$\frac{5}{8}$	$\frac{29}{57}$	51
B	$\frac{7}{13}$	$\frac{7}{12}$	$\frac{1}{3}$	$\frac{0}{2}$	$\frac{1}{2}$	$\frac{21}{42}$	50
C	$\frac{3}{6}$	$\frac{1}{2}$	$\frac{3}{7}$	—	—	$\frac{7}{14}$	50
D	$\frac{0}{3}$	—	—	—	$\frac{5}{6}$	$\frac{5}{6}$	55
4 large Rounds (percentage)	61	57	45	7	61	$\frac{62}{122}$	—
E	—	$\frac{3}{4}$	$\frac{0}{1}$	—	—	$\frac{3}{6}$	—
F	$\frac{1}{1}$	$\frac{0}{1}$	—	—	—	$\frac{1}{2}$	—
G	—	$\frac{4}{4}$	$\frac{0}{1}$	—	—	$\frac{4}{6}$	—
H	—	—	—	—	$\frac{1}{1}$	$\frac{1}{1}$	—
J	—	—	—	—	$\frac{0}{1}$	$\frac{0}{1}$	—
K	—	—	—	—	$\frac{0}{1}$	$\frac{0}{1}$	—
L	—	—	—	—	$\frac{4}{4}$	$\frac{4}{4}$	—
M	$\frac{2}{3}$	—	—	—	—	$\frac{2}{3}$	—
N	$\frac{0}{1}$	—	—	—	—	$\frac{0}{1}$	—
9 small Rounds	66	77	0	—	71	$\frac{16}{33}$	65
Condensed milk	—	$\frac{1}{2}$	$\frac{2}{3}$	—	—	$\frac{3}{6}$	60
Totals of each section	$\frac{22}{36}$	$\frac{30}{32}$	$\frac{16}{36}$	$\frac{1}{13}$	$\frac{21}{33}$	$\frac{80}{100}$	—
Percentage of houses attacked	61	62	44	7	63	—	53

TABLE XXXI (*continued*).

Quadrilateral District.

Milkmen or milk- rounds	Sections of the district				Whole district	Percentages of houses attacked
	I Houses (4-44)	II (45-107)	III (108-130)	IV (131-213)		
P	$\frac{8}{14}$	$\frac{8}{10}$	$\frac{1}{3}$	$\frac{7}{15}$	$\frac{24}{42}$	57
Q	$\frac{5}{7}$	$\frac{6}{10}$	$\frac{3}{9}$	$\frac{9}{22}$	$\frac{23}{48}$	49
P & Q (percentage)	61	70	33	43	$\frac{47}{90}$	52
E	$\frac{2}{2}$	$\frac{2}{3}$	—	$\frac{1}{1}$	$\frac{5}{11}$	45
F	$\frac{3}{7}$	$\frac{4}{11}$	—	$\frac{0}{8}$	$\frac{7}{26}$	27
R	—	—	$\frac{1}{3}$	$\frac{3}{14}$	$\frac{4}{17}$	20
S	—	$\frac{0}{2}$	$\frac{0}{4}$	$\frac{1}{8}$	$\frac{1}{14}$	7
F, R & S (percentage)	42	56	14	13	$\frac{12}{57}$	21
D	—	$\frac{1}{6}$	—	—	$\frac{1}{5}$	—
G	$\frac{2}{2}$	$\frac{1}{1}$	$\frac{0}{1}$	$\frac{2}{3}$	$\frac{5}{6}$	—
H	$\frac{2}{2}$	$\frac{1}{3}$	—	$\frac{0}{2}$	$\frac{3}{5}$	—
J	$\frac{1}{1}$	—	—	—	$\frac{1}{1}$	—
K	$\frac{3}{3}$	$\frac{0}{2}$	—	$\frac{1}{1}$	$\frac{4}{6}$	—
T	—	—	$\frac{0}{1}$	$\frac{2}{6}$	$\frac{2}{7}$	—
U	$\frac{1}{1}$	—	—	$\frac{1}{1}$	$\frac{2}{2}$	—
W	$\frac{2}{2}$	$\frac{2}{3}$	—	—	$\frac{4}{5}$	—
X	—	$\frac{0}{1}$	—	—	$\frac{0}{1}$	—
9 small Rounds	100	35	0	50	$\frac{22}{41}$	56
Condensed milk	—	$\frac{0}{1}$	$\frac{0}{1}$	—	$\frac{0}{2}$	—
Totals of each section	$\frac{29}{41}$	$\frac{25}{56}$	$\frac{5}{22}$	$\frac{27}{86}$	$\frac{86}{196}$	—
Percentage of houses attacked	70	44	22	33	—	43

of houses, and the incidence upon the sections as a whole was very low, obviously from the operation of other factors. The milk-round of *F*, in spite of its low incidence of 27 %, the lowest of any in Section IV, has still the comparatively large number of 7 attacked houses out of 18 in the heavily attacked Sections I and II. Again, the 9 smallest rounds showed a constant tendency to be fairly evenly affected. It thus appears that diarrhoea occurred alike in all milk-rounds, and had little to do with the precise distribution of the various milk supplies. On the other hand, it must be admitted, after making many allowances, that the rounds of *P* and *Q* showed a somewhat constant tendency, even in Section IV, to be somewhat more highly affected than the rounds of *F*, *R* and *S*; although not more so than the combined totals of the

9 smaller rounds: and it is noteworthy that a careful analysis showed that this is not satisfactorily explained by differences in the proportion of dirty houses, or of those sheltering susceptible children contained in these rounds; although accidental grouping of the customers in peculiarly affected areas will explain a good deal. It is also remarkable that though the combined diarrhoea incidence on the rounds *P* and *Q* on the one hand, and that on the rounds *F*, *R*, and *S*, on the other, are widely different, yet each of these combined incidences are found to vary regularly together, in the various sections, with that of each section of the whole district, as shown at the foot of the table; and also regularly at all times of the season with the variations in incidence upon the whole area (cf. also Chart VI, App.). Thus, if the high incidence on *P* and *Q*, or rather the low incidence on *F*, *R*, and *S*, had any significance, it must have been of a constant and general kind, present throughout the whole season, such as that attributable to different standards of general cleanliness in the houses of certain milkmen. The bacterial content might of course only act as a general predisposing influence to the intensity of the specific organism; local distribution being however decided wholly by the latter.

The even distribution of attacks throughout the season has been illustrated by the construction of Chart VI, App., where the distribution of attacked persons, as regards date of onset, is shown in the different milk-rounds through the season. Very complete analysis is given of the two largest rounds in each district, which were also those falling most under suspicion for excessive diarrhoea incidence.

The teaching of these charts is particularly emphatic. In both districts there was not the least evidence of clumping to suggest a run of cases due to casual admission of infection to a particular milk-supply; but the cases were scattered throughout the season, the outline of the curve always tending to conform to the curve for all cases. Nothing but complete passivity was exhibited in the relation of the source of the milk-supply to the occurrence of cases. On a review of the matter, the passivity was exhibited, firstly, in Table XXXI, in the tendency of all milk-rounds in each large area to receive an equal share of the total infection; secondly, as regards locality, in the similarly equal variation of incidence upon the different milk-rounds with the variation in incidence upon the sections in which they were contained; thirdly, in point of time, in Chart VI, App., in the even distribution of cases in each section throughout the season, in passive conformity to the general seasonal curve of all cases; and lastly, in the

apparent absence of any influence of the milk-supply in determining the formation of the characteristic clumps of attacked houses (cf. Sect. VII, p. 688). Several different milkmen generally served the houses in each clump.

The evidence obtained from these districts suggests then the abandonment of a belief that the cause of diarrhoea is to be attributed to the introduction of infection into the house in the milk-supply, unless it be assumed that cow's milk acts in a general way, and that diarrhoea infection, from some common bacterial contamination occurring during production or storage, is common to all samples of cow's milk. Some milks might of course get more of such pollution than others, and it might be seriously upheld that this is shown in Table XXXI in the quadrilateral area, in the higher incidence upon certain milk-rounds. It must be pointed out, however, that no explanation has been given, in this enquiry as to the milk-supply, of the mass of evidence accumulated under "Epidemiological Features" (Sect. VII, 2 (*a*)) relating to the characteristic local distribution of diarrhoea, and the evidently purposive grouping of attacks in households which adjoin or have certain peculiarities of age constitution and sanitary condition in common. The mere effect of variation of temperature upon the bacterial content of milk cannot of course explain the latter; and thus, since the main characteristics and cause of the epidemic are developed without relation to the milk-supply, the implication of the latter, even in a general way, seems to have very little to uphold it.

It would of course be contrary to experience to suggest that diarrhoea infection could not be distributed in the milk-supply, or that such does not occasionally happen. A small outbreak in the northern corner of the triangle may have been an example of this. The occurrence of 6 cases in houses 150, 152, 153 and 154, was remarkable from the fact that 5 of them occurred within 7 days of one another, and no others occurred in those houses at any other time of the season, or about the same time in any of their neighbour's. The occupier of house No. 150 was milkman *L*, who supplied the 4 houses, the only houses—unfortunately for this enquiry—in that milk round in the district. The milkman *L* contracted typical diarrhoea and may thus have been a means of infecting the others through the milk supply. Milkman *L* did not personally distribute the milk. It was stated, however, that diarrhoea was contracted, in Nos. 153 and 154, four days before the former was attacked. The dates were perhaps rather doubtful, and at that unsatisfactory point the enquiry must be allowed to remain.

Milkmen *A* and *B* also lived in the district, at 142 and 140 respectively ; they appeared not to have had diarrhoea during the season. Due allowance must of course be made for any history of that kind obtained from milksellers. In the quadrilateral one of the milk vendors, milkman *F*, lived in the district in a house behind No. 205 in σ St., but gave no history of attack in his household.

(c) *The suggested infection of milk within the home.*

Newsholme (1902 and 1906) at Brighton found that amongst infants dying of epidemic diarrhoea condensed milk was an even more potent source of infection than cow's milk, and that there was also a definite number who developed the disease when fed upon the breast alone. From both these facts he argued that "diarrhoea is mainly due to domestic infection." Sandilands (1906), with data collected at Finsbury, confirmed the two former observations, and also showed experimentally that condensed milk (Nestlé's) not only contained very few bacteria at the time the tin was opened, but that when left exposed to the air at ordinary summer temperatures there was hardly any increase of bacteria at the end of a week; in contrast to the enormous increase occurring in cow's milk, exposed under similar conditions. He suggested that the comparative potency of the two kinds did not therefore depend upon the bacterial content, that is, upon the total number of bacteria they came to contain. Moreover, since from Park (1901) and Delépine's (1903) experiments it might be fairly deduced that the bacterial content and related pathogenicity of milk varies directly with the height of the shade temperature; and since the number of cases of diarrhoea (mortality) are not found to maintain a constant proportion to the degree of air temperature, particularly when the ends and middle of the epidemic period are compared; he therefore concluded, for this reason also, that no direct influence on the incidence of diarrhoea was referable to the mere bacterial content of milk. Various reasons will however be given later (p. 746) why the absence of an absolute numerical relation of cases to the air temperature is not, necessarily, inconsistent with a direct influence of temperature upon the bacterial content of food; amongst which is included the possibility that the cause of the disease is limited to one specific organism, not of general distribution, whose dissemination at the beginning of the season is a matter of some little time.

As regards the special importance assigned to infection through milk, it can be readily understood how, when infection by food came to be accepted, the former had the misfortune to fall doubly, and possibly unduly, under suspicion, from being a medium so eminently suited for the multiplication of bacteria, and also from being so generally distributed as an article of food.

In the districts under consideration a number of enquiries were made as to the amount of milk taken per household, and as to how it was finally disposed of amongst the various members of the family. From this it appeared that amongst the older members of the household, amongst whom the greater part of the total cases occurred, uncooked milk was very seldom taken. The practice of giving drinks of milk, alone or with other food, to the younger children, as before mentioned, was very general; but what afterwards remained over was usually cooked in puddings. In preparing bread and milk the latter was as a rule first brought to boiling point. It would thus as a rule have been possible to exclude altogether the influence of unboiled milk, amongst large numbers of attacked adults, but for the practice of taking milk in tea, which was almost general. This question of milk in tea therefore becomes important. It is probable that, at the time the milk is added, the tea is as a rule at 160° F. or a higher temperature. If it should prove then, that this is sufficient to destroy the virus of diarrhoea, it can be confidently affirmed that a large number of adults contracted the disease quite apart from the agency of milk. With regard to young children, the high attack rate observed in those fed on condensed milk has been noted above; and in a former section (p. 675) allusion was made to the 5 infants attacked, or 20 % of those under 9 months old, fed wholly upon the breast; 2 of these being infected from the mother. In both classes of cases infection must have been contracted within the home, and not by introduction through the milk-supply. Cow's milk, moreover, even as a vehicle of domestic infection, can here be altogether excluded. It is worth noting that the large amount of dirt and dust of the household which must have been introduced upon the crusts of certain breast-fed infants, had, at least under 9 months, no deleterious effect. Finally, a good deal of evidence will be given in other sections as to the frequent occurrence of infection within the home, but not necessarily through the agency of milk.

The question of exposure of milk and food within the home is discussed under "Cleanliness of the Household" (Sect. V, 1, p. 656).

(d) *Some conclusions as to the influence of Milk, and the Milk-Supply.*

Observations.

Conclusions.

1. The possible general part played by the milk-supply as suggested by the unexplained greater general incidence upon rounds *P* and *Q* in the quadrilateral area (cf. p. 678).

2. The possible dependence of the greater diarrhoea incidence at certain ages upon the extent to which milk enters into the dietary of those ages (cf. p. 673).

3. The bulk of evidence as to the influence of the milk-supply indicated that the latter took no part in the causation and peculiar distribution of diarrhoea attacks, *e.g.* in the formation of the "time and place groups" (p. 679); thereby nullifying the importance of this supposed commonest vehicle of infection from an impersonal source (cf. p. 678).

4. Boiling the milk—generally—on receipt did not decrease the incidence of diarrhoea (cf. p. 671).

5. The proportion of "first cases" amongst those fed on the breast or on cow's milk, respectively, was about the same (cf. p. 674).

6. 20% of children, wholly breast-fed, were attacked. 40% of those attacked under 12 months were not having any cow's milk (Table XXX). Many adults took no milk, or only cooked milk, and yet were attacked (cf. p. 681).

These observations, both of very doubtful value, suggest that if the milk-supply exerts any influence at all in introducing infection, it is mostly of a general kind.

The part played by the milk-supply is thus either a negligible or at least a passive one, the milk apparently coming to act merely as a vehicle of domestic infection, just as any other food might do. The evidence obtained points to the greater part of infection being caught within the home.

Plenty of infection therefore occurs within the home when cow's milk is altogether excluded from the dietary.

2. *Water.*

The water supply of the districts was excellent; it was exclusively tap water, laid on to each house from the town's supply. The latter is derived from deep borings in the sandstone, the works being situated in the Sherwood Forest, several miles out of the town. Water is generally excluded as a possible factor in the causation of epidemic diarrhoea where the supply is unexceptionable in source and quality.

3. *Fruit and Solid Foods.*

Fruit, per se, is popularly held to be one of the commonest causes of diarrhoea; a belief which will not however stand the test of more than a superficial enquiry. There are a number of points of difference distinguishing the simple diarrhoea or irritative digestive disturbance following over-indulgence in fruit from epidemic diarrhoea: the absence of clinical signs of a specific affection, and the absence of related specific cases (cf. Sect. III, 1, p. 619): it has also been remarked that the maximum age incidence of epidemic diarrhoea does not correspond at all with the ages at which fruit is mostly eaten: moreover, the seasonal abundances of the common fruits, individually, and even collectively, do not correspond with the seasonal prevalence of diarrhoea. Thus, the first fruit of the season, cheap and plentiful enough for general buying, are strawberries. It was noted that in 1908 these first became generally available about July 1st. At this time diarrhoea had already appeared and commenced to increase in various parts of the town. Plums have always received special notice in this connection; but they did not come into season till several weeks later again; while strawberries were already out of season before the epidemic had reached its height. Any correspondences in the fruit and diarrhoea curves must therefore be referred to the fact that they are both seasonal phenomena, which though naturally unrelated, are referable in common to the influence of the cumulative seasonal temperatures. Nevertheless the fact should not be passed over that digestive disturbance due to over-indulgence in fruit, particularly when unripe or fermenting, may perhaps strongly predispose to specific diarrhoea; and that if left exposed to infection it may, just as any other food, become the means of introducing the disease (cf. also p. 747).

A matter might be here mentioned, which relates to some extent to the food-supply of the district: enquiries as to diarrhoea were not made in shops, rows of which occupied part of the frontages of the triangular district: apart from other reasons, there was the question as to whether the shopkeepers would be willing to furnish complete or reliable details upon such matters.

If milk be allowed to be frequently a vehicle of the disease, as the result of exposure to infection, the part played by *solid foods* generally must also be allowed to be important, since they are exposed under similar conditions. A great part of solid food is cooked, but so also was a great deal of the milk in the houses examined. In a number of adult cases milk in any form was excluded, except the small amount added to scalding-hot tea. In many of these, as perhaps in most adults, infection if it came by food probably came through some form of solid food. In conclusion it is probable that, apart from its suitability as a nidus for infection, no particular food has more influence than another, except in so far as it is more frequently partaken of, or is perhaps more often taken uncooked or left exposed to infection.

VII. EPIDEMIOLOGICAL FEATURES.

1. *Introductory: Theories as to causation of epidemic diarrhoea.*

In considering the nature, origin, and transmission of epidemic diarrhoea, it is of special interest, and also somewhat necessary, to note the evolutionary stages by which, as one of the last diseases to do so, it appears to have finally emerged from the nebulous theories of humours and miasmata, which in the evolution of epidemiological beliefs have been replaced in most infective diseases by the demonstration of the contagium vivum, and of its method of transference by direct transmission or by animal and other carriers. As regards *the nature of the disease* (see also pp. 667-8) the present conception of its specific character we owe largely to the monumental labours of Ballard (1887-8), who by demonstrating an extensive pathology and symptomatology, laid bare what appear to be the foundations of a typical infective disease. The evidence as to specificity has been very generally accepted, but the solution of the further question involved as to whether two or more diseases are not represented by these diarrhoeas of seasonal epidemic type, will probably depend upon the final demonstration as to the

precise nature of the ultimate cause. Again, whether or not that cause is bacterial, and whether the bacteria are of a saprophytic or pathogenic type, yet remains to be shown, or at least to be indubitably established. In the present paper the older theories of causation as to mechanical or reflex causes, or heat *per se*, will be regarded as superseded; and it will be assumed that the disease is due to the action of, or to an intoxication connected with the existence of, minute organisms, whether residing within or without the body.

Next, as regards *the source of infection*, a point of some significance is the long retention of theories embodying or tinged with belief in miasmatic influences though these have been practically abandoned in other infectious diseases. Undoubtedly the long observed correspondence between the prevalence of diarrhoea on the one hand, and meteorological conditions and conditions of soil on the other, has been mainly responsible for this. Ballard's researches (1887-8) in this part of the subject were not so complete as those regarding the real nature of the disease, and they happened to be interrupted at a stage when he had arrived at his conception of the dominant influence of the deep (four-foot) earth temperature. Accordingly he came, at that stage of his views, to attach great importance to "emanations" from the ground; and his "practical suggestions to sanitary authorities, based upon the foregoing results of the enquiry as to causation" (*ibid.* p. 7), are specially directed towards the protection of the interior of houses, and of food, the principal vehicle with him of infection, from these "telluric emanations"; or towards lessening their virulence by keeping the soil, by adequate paving and drainage, free from pollution. He therefore urges that "the whole surface of the earth beneath houses should be so effectually and uniformly sealed with impervious material, as to prevent any chance of emanations rising into them from the soil." Thus, with regard to the storage of milk, he says that "the dairy should be similarly protected from the rise of ground air," and that "the practice often adopted of storing milk on the ground floor of a dwelling house or in some underground cellar should be altogether discouraged." There is no doubt that subsequent observers laid undue stress upon the 4-ft. earth temperature theory, for some time unduly confining their investigations to the influence of the ground, although it is but just to Ballard to state that he insisted on his conclusions being regarded as provisional only, holding himself (*ibid.* p. 2) "at liberty in a future report to withdraw or qualify statements made under this heading"; and he appears to have privately expressed a view at another time that the 4-ft. record

was merely to be regarded as a register of the accumulated summer temperatures (cf. Parsons, 1910). A general impression has however grown up of late years that the prevalence of diarrhoea has no relation to the movements of the 4-ft. earth temperature, except in so far as both are the effects of some common cause.

In the paper referred to, the writer (1908, pp. 7 and 8) pointed out that, though it was true that the 4-ft. temperature rule could be shown to roughly apply in this country in seasons in which the temperature was still ascending at the moment of the diarrhoeal rise, yet, in seasons where irregular rises and remissions of temperature occurred with the diarrhoea sometimes rising from a falling earth temperature; and in cold seasons, where 56° F. might not be reached; as well as in hot countries, where the 4-ft. temperature does not drop in the winter below 50° F. (cf. Armstrong, 1905); the rule was quite inapplicable. The claim of the latter to special pre-eminence over more superficial temperatures must therefore be clearly forfeited.

On the other hand, it was demonstrated that the air temperatures, or superficial earth temperatures, could be shown to be *the really* important temperature influences; for when one or other of these temperatures was taken, it was found that a period furnishing a certain sum of accumulated temperatures always preceded the rise in diarrhoea mortality. And this rule was found to be elastic enough to suit all seasons, including the irregular and abortive types in which the 4-foot temperature rule was found wanting. Thus the raising of the sphere of causative agencies to—at least—*the surface* of the ground was justified. In recent years it has, however, been suggested that the sphere of causative influences should be still further raised, *i.e. above* the ground: that the effect of temperature (of the air) is produced by causing an increase in the number of flies; and that the latter are secondarily responsible for the rise in diarrhoea; the interval of accumulated temperatures being mainly occupied by the incubation and multiplication of the fly carriers. The possibility of direct infection from person to person is another method of transference that has also received some amount of attention recently.

A further discussion of the practical application to the question of diarrhoea prevalence of the 4-ft. temperature record, and its reputed close relation to the fall as well as to the rise of the epidemic, and to the fly curve, is to be found on pp. 733 and 735.

The most important theories as to the source and method of transmission of the disease may be concisely tabulated as follows:

(1) *Possible source or Origin of infection.*

(a) *From a source outside the human body: an impersonal source: e.g. a saprophytic organism of the soil: the source of supply of causal organisms being not necessarily dependent upon periodic renewal from actual cases of the disease.*

(b) *From a human or personal source: the causal agency being always handed on directly or indirectly from a previous case of the disease.*

(2) *Possible Methods of Transmission of infection.*

- | | | |
|------------------|---|--|
| <i>Direct.</i> | { | (i) Direct personal infection. |
| | | (ii) Direct infection from an impersonal source, as by dust laden with ground organisms. |
| <i>Indirect.</i> | { | (iii) Carriage by flies. |
| | | (iv) Carriage by food, drink, or by dust containing infection derived primarily from a human origin. |

It is important to note that several or all of the various factors here mentioned might play some part as regards both the source and method of transmission of infection. Thus the causal agency might prove to be derived from both sources (a) and (b); *e.g.* if it happened to be a saprophytic but facultatively pathogenic organism. Similarly it may be transmitted in one or all four of the methods above instanced.

These theories will now be successively tested against the evidence of the data gathered in the various districts of the town.

2. *The Evidence as to causation of Epidemic Diarrhoea.*(a) *The origin of infection from a human or personal source.*

It has been suggested that all diarrhoea infection is derived from a human or personal source, being transmitted directly or by means of carrier agencies from case to case, complete continuity of infection being maintained from season to season by the few cases which are habitually found to occur throughout the winter. It would of course be difficult, if not impossible, to completely demonstrate the truth of this theory, for that would necessitate a proof of human origin in

practically every case: evidence will however be given that quite a large part of diarrhoea infection owes its origin to passage from person to person.

The following 14 points deal at length with the evidence obtained in the two districts pointing to spread by house-to-house, and by case-to-case, infection. Reference should also be made to p. 715 *et seq.*, where they are briefly set out in tabular form.

(1) *There was a constant tendency for attacked houses to be found gathered together into groups or clumps of neighbouring houses.* This tendency, which has already been shown to occur to a great extent apart from the influences exerted by dirtiness and the presence of infants (cf. Sect. V, 1 (*f*), p. 646), is very evident in the charts of both districts. In the triangle there were 13 such groups or clumps, where complete rows of from 3 to 7 houses were all attacked. These were separated by correspondingly wide intervals of unaffected rows of houses.

(2) *Again, there was a constant tendency to grouping in point of time.* This should be carefully followed out in Chart VII, App., where all the most remarkable examples in the two areas are given. It should be noted that in most of these *time* and *place groups*, into which quite three-fourths of all attacked houses are gathered, few, if any, attacks occurred in the many weeks of the season preceding or following the period specially indicated; also that the period of affection of one small group might be many weeks earlier or later than that of another closely adjacent. Both of these facts emphasise the marked isolation of outbreaks in the respective groups from one another, and by contrast the very close relation in which the individual cases of a group stand to one another. As regards the explanation of this characteristic clumping of attacked houses, the suggestion might be made that it is due to a mere chance distribution; but even on taking the local grouping alone, its peculiarly well-marked character in both districts, along with the other evidence of house-to-house spread to be noted hereafter, considerably minimise the possible sufficiency of such an explanation: when, however, the grouping in point of time, as well as of place, is considered, the evidence for community of infection is quite convincing, and the question of chance is no longer admissible. Such community of infection might of course be interpreted as indicating continuous transmission of infection from one person to another; but possible infection of each person and house in the clump, separately, from a common source, as from a focus of ground infection, must also

receive due consideration. The milk-supply had apparently no influence in determining the formation of these clumps (cf. Sect. VI, 1 (b), p. 679). It is not improbable, therefore, that *the phenomena of diarrhoea prevalence are almost wholly concerned with the local evolution of various infective foci*; the disease not being distributed to the population in a general broadcast fashion, as by the water, milk, fruit, or food supplies. The exclusion of the latter factors leaves us then with but two possible sources of the disease to consider—case-to-case infection, and ground infection.

(3) The distribution of *backyards-in-common* in the two districts is described in detail under “yards-in-common” (Sect. IV, 4), as also other structural matters which tend to bring neighbouring households into close relationship. *Rows of houses with backyards-in-common appeared to be particularly liable to be affected together, or to escape together.* This is most apparent in the triangle, where common backyards were found throughout. Houses 6 to 40 in α and β Streets are practically made up of four such rows wholly affected and three wholly unaffected. The same tendency can be followed throughout on the chart, in each of the other streets.

Table XXIV shows that in the triangle there were 179 houses having backyards common to two or more houses, there being 50 of such backyards in all, common to from 2 to 15 houses, and averaging nearly 4 houses per common yard. The distribution of attacked and unattacked houses with respect to these yards was very remarkable, being as in Table XXXII below:

TABLE XXXII. (*Further details are given in Table XXIV.*)

Triangular Area.

			Backyards-in-common having the houses		
			More than half attacked	Half attacked	Less than half attacked
Number of yards	21	9	20
Average number of houses attacked per yard, as a percentage.			77	—	15

Far from there being, upon summing up, an almost equal distribution of affected houses per average yard, there was an average of nearly eight houses out of ten affected in half the yards, and in the other half about eight out of every ten were unaffected. In other words the division into yards had apparently an extremely important influence upon the distribution of infection.

Such a conclusion must have far reaching practical issues; and although the available data are limited in amount, a detailed enquiry is warranted before passing on.

It might be suggested that, given the characteristic alternation of groups of attacked and unattacked houses, apparent also in the quadrilateral notwithstanding the almost complete absence of yards-in-common, the unequal distribution into low and high incidence yards is only the necessary result of a mere chance arrangement: but the question of chance is largely excluded by the fact that the yards are of different lengths; some are just as long as the longest clumps; and there is a distinct tendency for long clumps to be found in long yards and short clumps in short yards. An extension of infection to the house immediately adjacent, in the next yard, occurs in three instances, and to two houses in one other instance; but on taking in these houses or the yards they are contained in, it will be found that the high incidence yards now come to contain the whole of the affected "clumps" of the area; leaving, in sharp contrast, a series of practically unaffected yards distributed alternately amongst them. While there can be no doubt of the marked limitation of the disease to yards-in-common, it is not so clear as to whether this was not largely due to the fact that the boundaries of these yards determined differences in structure and date of building of the houses; differences in rent (cf. Table XVI), and therefore also in the class of people; in household dirtiness (cf. Table XXVII *b*, App.); and in the numbers of houses containing infants (cf. Charts I and II, App.). Thus of the largest yards containing four or more houses, the 11 largest high incidence yards contained twice as many infants (under 2 years) per yard as the 9 largest low incidence yards. However, four of the first group and three of the second had respectively low and high percentages of infants. Thus in 7 out of the total 20 yards the incidence was determined in direct antagonism to the influence of houses containing infants. Similarly as regards dirtiness, infection frequently failed to cross the yard boundaries into dirty and presumably susceptible yards, *e.g.* houses 31—35. Low incidence yards whose immunity cannot be accounted for except by the limiting influence of yards include the following houses: Nos. 31—35, 36—38, 64—66, 98—101, 131—135, 136—139, 159—162, 170—177 (these latter have yard entrances separate from the houses at the other end of the yard; an almost complete division should therefore be recognised). The failure to spread between the following is also worthy of note: between Nos. 19—22 and 23, etc.; and 41—45 and 46, etc. On the other hand most of

the high incidence yards showed remarkable immunity, but at the same time striking limitation of infection, *e.g.* the three yards on the east side of γ Street; and notably the yard of Nos. 41—45, where the wholesale involvement of a whole row failed to spread to neighbouring houses, which, though clean, contained numerous susceptible infants. Finally, on even a casual inspection of the charts, the influence of yards, though difficult to express in exact terms, is undoubted; and for further details reference should be made to the data there provided, and also to Table XXIV. It has already been remarked in connection with the latter table that the influence of yards-in-common in the triangle appeared to act even more powerfully in determining the distribution of the disease than the arrangement of houses containing infants or than dirtiness. The material is of course limited in amount, and the conclusions must therefore be accepted in a somewhat tentative manner.

Other details in the arrangement of neighbouring houses having special relation to the closeness of human intercourse. The structural arrangements, leading to the formation of a deep recess between the rear parts of successive pairs of adjacent houses has been previously described in detail (see Sect. IV, 4 and Chart III, App.). It might be expected that, owing to the limitations and specially close relationship as regards human intercourse and fly influence so established between each pair of houses, there would be found to be a greater incidence upon pairs of adjacent houses with their rear premises thus turned towards one another into the same recess, than upon pairs of adjacent houses whose rear entrances faced away from one another into two different recesses. To test this matter a tabulation of the houses in both districts was made, first of all as to the number of complete pairs present of houses with rear premises facing one another, and secondly as to the number of complete pairs present with rear premises facing away from one another. The incidence of diarrhoea with these alternative classifications was found to be as follows:

Of 328 houses arranged in complete pairs of adjacent houses with rear premises facing *towards* each other, 94 houses were affected in pairs, and 69 were singly affected.

Of 336 houses, arranged in complete pairs of adjacent houses with rear premises facing *away* from each other, 92 houses were affected in pairs, and 74 were singly affected.

Thus the proportion of houses in the first group affected in pairs was 57·3% of all affected houses in the group, and that in the second group 55·3%; a very insignificant difference when the rather greater

number of total houses in pairs in the second group is considered. The corresponding percentages in the triangle were 59·7 and 59·7, and in the quadrilateral 55·1 and 51·6 respectively.

The differences in the distribution of babies in the respective groups could not have affected the results very much; there was no difference in the case of the quadrilateral; and in the two districts combined, the number of houses with infants (under 2) in the doubly affected pairs of the first group were slightly smaller than in those of the second group, the proportion being as 40:44; a fact which rather tends to emphasise the difference in favour of extra incidence upon houses with their rear premises turned toward one another.

The smallness of the difference however, found between houses whose relations are qualified in the manner above described, rather detracts from the supposed importance of direct personal infection and fly carriage; unless it be supposed that flies have rather the habit of frequently passing backwards and forwards along extensive terraces, than of confining themselves for days together to one kitchen, or between the kitchens of the two houses facing into one recess; or again, that the playing together in the common yard of the triangle, or the frequent visiting between neighbours in the quadrilateral, overrides the importance of mere adjacency of houses or the sharing of the common recess, as regards the question of direct personal transmission.

The apparent limitation of the disease to common backyards may however be interpreted as being opposed to extensive movements of flies; or, on the other hand, as emphasising the importance of direct personal infection, such yards presumably limiting human intercourse much more than the movements of flies.

Two possible explanations of the special importance of common backyards persistently obtrude themselves upon one's notice. Firstly, the constant faecal pollution of the yard surface where there are young children, particularly frequent when they are affected with diarrhoea. The yards in the triangle, at least immediately around the rear premises, were asphalted; and the way in which such impervious pavement, if in the charge of dirty people and if left unswept and unwashed, helps really to conserve infected faecal material and disseminate it as dust, has already been commented upon (see Sect. V, 2, p. 657), as well as the fact of the encouragement its warmth may possibly give to fly carriers. Secondly, there is the close association, playing together, and constant visiting from door to door, especially of the young children of the yard. In the triangle the common yards, where the high incidence was most

striking, had very little depth—or at least this was true of the part to which the children were restricted. The three long yards on the east side of γ Street may be taken as an interesting example. They were only about 12 feet in depth, between the rearmost part of the houses and the high back fence; and were asphalted throughout. These yards were kept in a dirty state and at practically every visit faecal material was noticed somewhere upon the pavement, the dust of which must therefore have been highly saturated with diarrhoea infection. What more natural than to expect that, on this account alone, many of the 24 children under five years of age belonging to the 18 houses of the terrace, who were constantly playing together in this confined and highly infected space, should develop diarrhoea! Such a degree of pollution with typhoid stools would doubtless be regarded as sufficient cause for a large typhoid outbreak. It is evident then that with regard to yards also, everything depends upon the dirtiness or cleanliness of the people's habits.

Whatever may be the real influence of yards, it is at any rate evident, from the marked clumping of attacked houses, to be found also in the quadrilateral, that this latter characteristic method of distribution of the disease is the feature of prime importance and holds good either in the presence or absence of yards-in-common. It should be noted however that it was perhaps on account of the influence of backyards, as well as of the irregularity in response to the differential influences of dirtiness and of babies, that this clumping in time and place was more marked in the triangle than in the quadrilateral; and again, it may have been on account of the absence of yards-in-common in the latter district, that the dates of the grouped attacks were further apart, as if infection had greater difficulty in passing along between neighbouring houses.

In order to connect up these observations with those of previous sections, it might again be repeated that these time and place groups of attacked houses were generally confined to a row of houses of small extent, and were frequently distinctly marked off from other similar neighbouring groups. Moreover, only on three occasions—in α Street, and in π and ρ Streets—did houses on both sides of the street, or rows of houses immediately behind, or otherwise adjacent, appear to be included in the same group. Thus, if fly carriers are the cause of spread, they appear to ply most of their time between just a few houses; streets and extensive back gardens both offering considerable obstruction to the spread of the disease.

The general impression gained then from the study of all these facts is that the disease has typically a tendency to distribute itself in a number of scattered foci, formed of clumps of adjacent households. Although these groups or clumps are usually rather smartly involved—several houses of a clump being generally invaded within the first two or three days, and the whole outbreak being practically over in two or three weeks (cf. Chart VII, App.); yet, it is not often that more than from three to eight houses are included, infection creeping outwards from such a focus in a rather forceless way, and being apparently easily obstructed by such natural obstacles as streets or unoccupied spaces. The large number of scattered cases, found in addition to those included in the characteristic clumps, may of course be attributed to actual human convection or to fly carriage from a distance. It might here be noticed that all that has just been said of both the clumped as well as of scattered distributions might be interpreted as pointing, just as favourably, to infection from a ground origin as to infection from a personal origin.

(4) The manner of the appearance of related cases of diarrhoea is very similar to that observed in scarlet fever and diphtheria; *the cases not occurring simultaneously, but generally following each other with at least some small interval between*, and not infrequently with intervals of several weeks between each. The marked tendency of the disease to follow a chronic course (Sect. III, 3), and the possible long retention of infection with recurrence of attack after apparent recovery (Sect. III, 4) may be here referred to as doubtless explaining the infection of long series of cases not always apparently connected.

The cases then do not occur in crops, as in measles and chicken-pox, except in so far as a sudden rise of temperature following a period of cool weather in the middle of summer will simultaneously bring on a crop of cases in many neighbourhoods where a few smouldering cases have been already established. By contrast with the diseases mentioned above, the absence of crops points to an incubation period of short and variable duration; and again to the derivation of infection, not from a common source, as in the accidental admission of infection into the common milk or food supply, but by successive passage from one individual to another, whether by direct or indirect means.

In the charts, it must be admitted, there are more than a few instances to be seen in which infection was said to have occurred on the same day, in two or more cases, in the same family or in one of the associated groups of families. Exact dates, however, could only be obtained in a

minority of the cases. It was customary to put down the same date for cases of which no more definite statement could be obtained than that they occurred "about the same date." The frequency of multiple cases occurring on the same day is therefore overstated. There were however a number of cases, of recent occurrence, where fairly definite dates could be obtained. On extracting all of such cases, it was found that in Chart VII, App., there was only one instance in which more than two cases occurred in the same group on the same day, and 13 other instances in which two cases occurred in the same group on the same day. In five of the latter, the two cases occurred within the same household. The frequency of cases occurring on certain days, *e.g.* Sundays, Wednesdays and Thursdays, will however be shown to have been overstated. Six out of the above-mentioned 13 instances occurred on Wednesdays and Sundays, and a doubt must therefore be admitted as to whether the true date was always given (*cf.* p. 739).

Another fact suggesting the passing of infection from case to case as opposed to ground infection, is that in more than half of the time and place groups of Chart VII, infection appeared not to spring up spontaneously, but to be due to sudden dissemination around some antecedent case or cases that had been going on quietly for several weeks preceding.

Again, the time and place groups in Chart VII, just as the individual cases, are seen to be scattered over the whole season; differing widely in point of time, even in adjacent neighbourhoods. Transplantation of infection from a personal source is thus suggested. Reference, upon these points, should also be made to p. 737 *et seq.*

(5) *On some occasions the epidemic appeared to perambulate a locality*, gradually passing down a row of houses or a street, successively affecting the various houses in its path and appearing to use up the susceptible material as it proceeded (*cf.* p. 739 *et seq.* and Charts V and VII, App.). It could not however be proved with certainty that this appearance was not due merely to an accidental successive development in neighbouring foci. Besides this perambulation of streets, there was some appearance of spread of high grade infection from one large district to another, the main outbursts thus occurring at different times in different districts.

(6) *The groups or clumps of attacked houses were distributed in a somewhat capricious manner*, often without regard to the degree of dirtiness, the numbers of susceptible infants, the degree of immunity, or other factors recognised as largely determining the local incidence of diarrhoea. Such eccentricity of distribution is regarded as particularly

characteristic of an infectious disease, since it must almost certainly be determined by chance transplantation and actual personal conveyance of high grade infection (cf. pp. 646, 695 and 737).

(7) Infection of a house or group of houses was in some cases traced to the *introduction of the disease by a person who had contracted it in another part of the town*. A good illustration of this was given in the group of houses Nos. 145—7 in the quadrilateral—situated in that block between σ and τ Streets already referred to as consisting almost wholly of four well-marked and distinct groups of affected houses. The first group in this block to be attacked were houses Nos. 145—7. The father in 145 first developed it, having violent diarrhoea and being confined to bed for a fortnight under the doctor's care. His attack was associated with a similar serious outbreak in his father's family, living in another part of the town, where five attacks had occurred and the doctor had been called in; infection of this latter household also requiring medical attention, and being again derived from an earlier outbreak amongst neighbours two doors away. The father at 145 had been a frequent visitor to his father's family. His illness was followed by affection of his wife and baby daughter and of his two neighbours' households, Nos. 146 and 147, within the next fortnight. In all, seven individuals were attacked in the three houses mentioned. There can be no reasonable doubt of the connection between the two foci—that around 145 in the quadrilateral and that around the father's house in the other quarter of the town, since the outbreaks occurred in the middle of June, when only scattered cases were as yet to be found over the town, and none had so far occurred in the block between σ and τ Streets. There was not opportunity of tracing out the origin of infection of the other four groups of houses in this block, but the interdependence amongst the individual houses of a group as regards the origin of their infection is nowhere so strongly suggested by the grouping of cases in point of time and place, as here. Infection was introduced into No. 135 by the mother, who frequently nursed her neighbour's child in 134 while suffering from diarrhoea (see p. 701), and apparently by that direct means contracting the disease herself.

(8) *The immediate invasion of a number of new houses* in the quadrilateral, built on clean meadow land and occupied as soon as finished, frequently before the walls were dry, is particularly significant of infection from a personal source, and opposed to a theory of ground infection. Again, a number of persons developed attacks immediately after coming to live in houses within the districts.

(9) *The large amount of multiple infection occurring within the household* has already been discussed in Section II, 1 (b). A comparison of the amount of multiple infection occurring in scarlet fever is instructive. While in these diarrhoea data 47% of all houses had more than one member attacked, only 20% of households were found to have more than one attacked in the case of scarlet fever, for the five years 1904-8, at Nottingham (Annual Health Reports). It is at least legitimate to deduce from this comparison that the amount of multiple infection in the household in diarrhoea is quite as remarkable, if not more so, than that of diseases in which transference from person to person is the chief means of transmission.

(10) Important statistical evidence has already been produced as to *considerable transmission of infection* to older members, *within the household*, by young persons of a highly susceptible age (cf. Sect. II, 1 (b)): also the tendency to spread has been found to be increased within careless and dirty households (cf. Sect. V, 1 (h)).

(11) *A masking or smothering of distinctions, due to dirt and to other factors* largely determining the liability to diarrhoea, has been shown to occur *within the clumps* of attacked houses, or in areas of high diarrhoeal incidence. It is not improbable that these differential factors are here overruled by the influences, apparently more powerful at such close range, tending to house-to-house spread (cf. Sect. V, 1 (f)). But the possible influence of a focus of ground infection cannot be altogether excluded.

(12) *A very much greater incidence* was found upon all members *within households* situated in *high incidence clumps* or sections, than upon those in low incidence sections (cf. Table XXV). This probably means house-to-house spread.

(13) *The mass influence* exerted by collections of closely adjacent *houses containing infants* (cf. Section V, 1 (g), p. 651), in definitely increasing the incidence upon all houses with infants within their neighbourhood, *necessarily points to the passage of infection from house to house, and from case to case*. No other interpretation meets the case.

(14) A large amount of evidence was collected (see Section VII, 2 (b) following) as to the frequent occurrence of *cases where it appeared almost absurd to suggest any other method of transmission* than that of *direct personal infection*. *Winter cases* must almost certainly be of the latter class.

Presuming that infection is generally derived from a personal source, the possible methods of transmission, by direct personal infection, and by fly-carriage, must next be considered.

(b) *Transmission by Direct Personal Infection.*

While the general question of the communicability of diarrhoea has of recent years come into some prominence—mostly as the result of observations as to multiple infection and from the attempt to link up the fly carrier theory, the possibility of *direct* communicability, or direct personal infection, has seldom received more than merely tentative notice. Upon a close search of current literature, it was found that when the question of communicability of diarrhoea was mentioned, there was seldom any recognition of the indirect and direct kinds of communicability, or at least any attempt to indicate what kind was meant. This is probably owing to the fact that mere communicability was itself till recently a matter of general doubt.

Johnston (1879, p. 204), however, in quoting his personal experience of infection while examining diarrhoeal stools, along with the observation that amongst 3,318 cases of diarrhoea applying for medical relief at Leicester, 20 % were associated with from 1 to 6 cases in the same house concludes as follows: these facts “have convinced me,” he says, “that summer diarrhoea is contagious and that the chief vehicles of the poison in the above instances were the ejecta from the bowels of previously infected persons.”

Ballard (1887–8, pp. 7 and 18) makes but very little reference to communicability in diarrhoea, and that in a somewhat tentative manner. He says, “communicability through the medium of the morbid evacuations does not appear to be a character uniformly attaching to the disease.” “In proof, however, of the occasional communicability of an (*sic*) epidemic diarrhoea” he refers the reader to four small outbreaks, which he describes as of the class “not distinguishable from epidemic summer diarrhoea” recorded by Dr Bruce Low in the appendices of the Report (*ibid.* p. 127). In referring to such outbreaks he on one occasion uses the expression “directly communicable.”

In a preliminary notice, in 1908, as to the results of the present enquiry, the writer (1908) stated that they pointed to the conclusion “that the bulk of cases are caused by case-to-case infection” (*ibid.* p. 17): also, that while “he was not impressed with the all-importance of milk, he did see a great deal of evidence as to the importance of direct personal infection” (*ibid.* p. 56).

Evidence may now be put forward, relating to the question of direct communicability, and illustrating the nature of the observations upon which the possibility of such an occurrence rests. Reference

might first be made to certain parallel occurrences observed in both diarrhoea and typhoid fever and pointing to direct transmission of infection. In view of the fact that both these diseases are lodged particularly in the alimentary canal and have numerous other points in common, it is of some importance to note that the belief has gradually come to be adopted in the latter disease that quite an appreciable part of infection is contracted from direct association with the patient himself. Actual personal experiences of typhoid fever, such as those upon which this belief is based, may here be related by the writer, for comparison with similar occurrences to be instanced in the case of diarrhoea. These include instances of the former disease in a non-typhoid ward attacking a patient in the bed adjoining what was afterwards proved to be a case of undoubted typhoid, when contamination of feeding vessels and actual personal contact could be excluded; as well as attacks of non-typhoid patients in typhoid wards, and attacks of numerous attendant nurses notwithstanding their special training as to how to avoid the risk of infection. Reverting to diarrhoea, the question at once suggests itself, Are not such occurrences strictly comparable to the occasionally reported instances of diarrhoea spreading in an infirmary ward amongst the infant patients? Another personal recollection may be added, of one of the not infrequent instances of typhoid fever occurring in an attendant whose daily duty it was to empty typhoid pails. Such, again, are almost certainly exactly analogous to similar recorded instances occurring in diarrhoea. Thus, Bruce Low (1887-8, p. 127 *et seq.*) mentions, for example, in describing the outbreaks discussed further on, several cases in which infection followed upon mere presence in a room where diarrhoeal motions were being passed or had been voided on the floor: Johnston (1879, p. 204) again relates the curious personal experience of being himself attacked on five different occasions within a month and a half, while making a prolonged microscopical study of the faeces of diarrhoea patients.

Turning now to accounts of actual outbreaks of the disease, Bruce Low's (1887-8, p. 127 *et seq.*) records of the four outbreaks referred to above, occurring in small villages of Yorkshire, are well worth reading at length, and must be here briefly quoted. One of these occurred in summer, at the end of August 1886, and was directly traced to infection from a typical case of summer diarrhoea imported from Leeds—"British Cholera," the neighbours said, was then very prevalent in Leeds: a series of 60 cases followed in the village. The diarrhoea

epidemic in Nottingham, during the same season, reached its acme in the middle of September, and reached winter level at the beginning of November. The three other outbreaks occurred in the months of December, February and May; that is in winter or early spring, when the influence of fly-infection, in two at least, could be altogether excluded. There were 180 cases in the four outbreaks and in all four the symptoms were precisely those of typical epidemic diarrhoea and the manner of infection was apparently the same. In a large number of cases it could only be attributed to the person attacked having been present in a room while diarrhoeal motions were being passed. In practically all cases there had been this history, or that of having handled soiled napkins, or of having used a privy after a diarrhoea patient. The attacks generally occurred during the night or day following such exposure to infection.

Similar instances suggesting direct communicability, collected by myself during 1908 and 1909, will now be given. The sixth occurred in the winter, and the influence of flies could be excluded; the others in the diarrhoeal season. When not otherwise stated, it is presumed that food and other common channels of infection could be excluded.

(1) The most remarkable case was where infection spread amongst the five occupants of two railway signal boxes set upon a breezy situation about a mile apart: infection was clearly carried between the boxes by a relieving man, and all five men were attacked; families of men in each box being also subsequently affected. Two of the men were newcomers, and each was attacked within a week of his arrival. There was no community of food or drink; the latter were always brought from home, and were protected up to the moment of eating. There appeared to be apparently no opportunity for fly-infection of the food; and the only reasonable explanation afforded seemed to be their close association in the crammed quarters of the box for ten hours a day, with the constant handling of the same apparatus; or the sharing of the common pan closet which was placed at a distance of about 30 ft. from the box (cf. Bruce Low, 1887-8).

(2) The next is a case of two sisters who went away together on a holiday, one of whom developed diarrhoea immediately after leaving, but the other not for several days after, and therefore most probably not from the original source from which the first sister was infected. It seems most probable that the second sister had the disease passed on to her by the first, who slept with her, or by a friend who was suffering from a severe attack of diarrhoea when she visited her for

a few hours, just 24 hours before her own attack, and by whom she was kissed on leaving.

(3) In a third instance a little girl aged two years was taken in to play, for an hour or two, with a neighbour's baby, who had a severe attack of diarrhoea at the time. This was at 7 p.m. At 3 a.m. next morning she was taken ill with severe diarrhoea which lasted two days, apparently communicating it directly afterwards to several other members of the family, who up till then had not been attacked.

(4) A married woman, who was herself childless, was in the habit of lifting her neighbour's baby over the intervening wall and nursing it. An attack of diarrhoea in the latter was followed, before its cessation, by a typical illness in the former.

(5) A mother was attacked with vomiting and diarrhoea about midnight; and her baby, aet. three months, developed a fatal attack of diarrhoea shortly after midnight of the following day, "dirtying the bed." The child had been taken off the breast two or three weeks before, and put upon barley water and milk, boiled and intelligently prepared with scrupulous care.

(6) An instance of a collier and his working mate contracting diarrhoea, within one or two days of each other, and where there was no community of food, is—at least—somewhat suggestive.

(7) A little girl, aet. 2, was attacked with diarrhoea and vomiting in January, 1909; and the only neighbour, a spinster, aet. 60, had suffered from a similar attack for a few days previously, during which time she had mended and returned a parcel of clothes to the family of F. 2. At the end of a fortnight, the latter family moved to this town, and the baby, F. 4/12, developed a similar attack, her death about two weeks afterwards being certified as due to "Gastro-Enteritis." Case-to-case infection is here undoubted. "Colds" however took a prominent part in each of the three attacks.

(8) The writer had a personal experience of the disease, apparently contracted while making a prolonged investigation into the second of the above recorded instances. Perhaps an hour and a half was spent in the sitting-room: both of the attacked persons above referred to were present, and one of them was still suffering from diarrhoea. No food was taken, and no flies were noticed. Diarrhoea commenced about 30 hours afterwards and continued for 4 days. Although a mild attack, it was interesting to note that apart from its duration and the mucous stools, the settled abdominal pain and accompanying sinking feeling left no doubt as to its specific nature; these were in

marked contrast to the symptoms of several experimental attacks of simple diarrhoea, induced by excessive indulgence in fruit. Home infection could not, with any certainty, be excluded. There was no recollection of a previous attack: but recollections of this disease are seldom reliable (cf. p. 622).

Before proceeding to the discussion of this evidence, it would be well to first define *what is the precise meaning of the term direct personal infection, as used in this paper*. It is of course a very arbitrary term, and both here and as generally used it is made to include all those cases in which infection through the recognizable channels of milk, water, and food supply, obvious gross contamination with infectious matter, and fly-carriage, can be excluded; leaving no assignable cause but the close association in which the person attacked has been with another suffering from the disease. There is, again, no real line of distinction between this and other methods of infection, as long as these two rather essential conditions as to close association with an infected person, and the tendency to invisibility or apparent unexplainableness of action, are fulfilled. Thus, by analogy with typhoid fever, the typically direct method of infection in diarrhoea might be held to be by the intaking of minute infected particles of dust, or spray, arising in the immediate vicinity of an infected person; the latter being derived from the splashings of liquid stools. But infection following actual bodily contact must also certainly be held to be of the direct kind, the infection in this case following directly upon contact through the hands or even through the clothing. Again, even the usually indirect channel—through food—may come to be considered as a direct one, in the case where infection results from coming immediately from attendance upon a patient and sitting down to eat with hands imperfectly freed from all traces of infection. The above are probably the chief methods of direct transmission in both diarrhoea and typhoid fever; the analogy with the latter disease is justified by the large amount of evidence already brought forward as to infection in diarrhoea occurring largely through the stools. It may be objected however that, in both these diseases, except for a few odd cases resulting from careless handling of the stools, there is practically no occurrence of true direct personal infection, but that every case of so-called direct transmission is to be assigned to the commission of some definite sanitary error which has escaped notice, such as laxity in the washing of hands, in the disposal of faecal matter, or in the protection of food from infected dust or from flies. It is of course as easy to make this assertion as it is

difficult, or impossible, to dispose of it by satisfactorily excluding all these possible channels of infection. Infection through the hand, however, may be considered in a large number of cases as strictly a direct means of infection; and from the constant communication of hand with mouth, it is doubtful whether, even in scarlet fever—an accepted type of diseases directly transmitted—more infection is not so introduced into the mouth than is inbreathed. Again, in diarrhoea, in view of the very common habit amongst young patients of passing their motions in the living room, and of the incessant pollution of the person, and of the floors, bedding, and clothing, there is, probably, as great a possibility of the inbreathing of infected dust or of minute liquid particles, as in the case of scarlet fever.

There is however no need to further pursue these theoretical considerations. As a matter of fact, the whole matter can be completely and clearly put in the following practical question, as applied, *e.g.* to the risk of infection from an ambulatory case of typhoid fever—Can infection be caught from such an ambulatory case in the ordinary associations of home life, where no specific preventive measures are taken, but where just the usual rules of cleanly living are observed, and apart from infection through fly carriers or any of the indirect and recognizable channels of infection, such as the milk, food and water supply? The answer to this query would no doubt be in the affirmative. It is proposed, then, to submit exactly the same question with regard to diarrhoea, and to speak of transmission of the disease, occurring in such circumstances, as direct personal infection.

Certain practical difficulties attend the satisfactory demonstration of direct personal infection: firstly, the demonstration must necessarily be one of an almost purely negative character, resting upon the satisfactory exclusion of all other channels of infection. And secondly, it must be based, apart from cases occurring under medical surveillance in hospitals, upon histories like those given above, gathered under the notoriously difficult and frequently unsatisfactory conditions of house to house enquiry. Collected in these circumstances, final proof of direct infection must be looked for rather in the dimensions of the accumulated mass of such instances than in their completeness of detail in individual cases.

Conclusions. The chief conclusions are set out in the following brief paragraphs:

(1) The question first arises, *with regard to the instances of infection recorded above, Are they all to be properly included as instances*

of direct infection, and of the typical epidemic form of diarrhoea? Exception might perhaps be taken, upon the latter point, to the instances described by Bruce Low, on account of their great infective virulence. There were, however, no essential differences to be found in the details of the various outbreaks, and one was distinctly traced to an ordinary typical case of summer diarrhoea. Moreover, the outbreak at No. 145 in the quadrilateral area, described above (p. 696), appeared to be just as virulent as any of these, as regards the severity of individual attack and the thoroughness with which it swept the several households involved. An analysis of Charts I, II, and VII, App., shows that the outbreak in the two districts was in great part made up of small outbreaks or small foci of infection such as the above. Perhaps the only difference between these and the village outbreaks referred to, lies in the fact that the latter occurred in a small community where a virulent local outbreak could show up to the best advantage, display clearly typical consecutive case-to-case infection, and have the best chance of preserving its individuality and the sharpness of its boundary from intercrossings with other centres of infection; whereas, in a large town, from the early appearance of numerous centres of infection, and from the constant intercrossings of infected individuals, this individuality and sharpness of boundary can seldom be made out. The great difference in virulence of different strains and centres of infection is referred to later on.

(2) As regards the precise value of the above-recorded instances, as evidence for the occurrence of an appreciable amount of direct personal infection, *their value depends very largely upon the frequency with which such occurrences were met with* in the districts canvassed. A limited number of them might always be fairly attributed to mere coincidence, being referred to unrecognised cases of fly carriage or other indirect infection. On the other hand, such clearly traced instances as the above are not met with so frequently as might be imagined, even in diseases such as scarlet fever and diphtheria, owing to the considerable intervals, just as in diarrhoea, that frequently separate related multiple cases. As a matter of fact, after making all allowances, the numbers and circumstances of the instances of the kind referred to were sufficiently remarkable to strongly suggest direct communicability. Finally, it is important to note all the surrounding circumstances of infection in the above instances; these were so convincing as to make it appear *almost absurd to seek any other method than that of direct transmission*.

(3) *Fly carriage is a factor peculiarly difficult to exclude in a*

demonstration of direct personal infection, from the almost complete parallelism of the conditions favourable to both. Both are favoured by closeness of association, but in both, notwithstanding, infection may leap over considerable distances, owing to the peregrinations of the flies, or again of the infected individuals. Both are again almost equally characterised by apparent invisibility and unexplainableness of action. The fly in its ceaseless journeyings to and fro must, in the course of the day, penetrate to almost every nook and corner of the house, thus establishing, quite unperceived, continual contact between every person and object in the household. Of course, a demonstration adverse to fly carriage would at once range a huge mass of doubtful cases upon the side of direct personal infection. It is evident from this to how great an extent advance in the knowledge of the disease and in practical preventive measures depends upon the speedy solution of this question of fly carriage. *In the winter time*, however, flies are necessarily excluded; and from two of Dr Bruce Low's outbreaks, and from several groups of related cases, I discovered in the winter of 1908-9, we must conclude that to a certain extent, at any rate, direct personal infection does undoubtedly occur in diarrhoea.

(4) The evidence as to limitation of infection to *backyards-in-common* (Section IV, 4, and p. 689 *et seq.*) must be held to favour personal infection rather than fly carriage, as these yards restrict the movements of persons rather than of flies.

(5) Instances such as those mentioned above, where *mothers have contracted the disease from infants* within 24 hours of their soiling the bed, and where *infants have been infected from mothers*, practically preclude the probability in these cases of infection being conveyed by other than direct means.

(6) *The ample opportunity of direct infection occurring from the constantly polluted bedding, floors and atmosphere of living rooms and bedrooms* has already been referred to (Section V, 1 and 3). The question might be asked of typhoid fever: How could the members of a family expect to escape infection, even in winter when flies were absent, if the whole household were impregnated with typhoid stools in this wholesale manner?

(7) *As regards the extent to which direct personal infection occurs in diarrhoea* two facts may be held to show that it plays a subordinate part: firstly, there is the very low prevalence in winter time when flies are absent, in some contrast with that found in typhoid fever: it will, however, be suggested later on (p. 748) that this may to some extent be

an exaggerated epidemic effect; and the tendency of diarrhoea to show a marked uprush and subsequently to exhibit intense seasonal exhaustion (cf. Chart B, p. 720, Melb.) must be noted as in strong contrast to the more leisurely rise and continued prevalence of typhoid fever. Secondly, in the data collected, there was an absence of facts suggesting the passage of any great amount of infection amongst adults at work or amongst school children (Sect. IV, 2 and 3). On the other hand, the great degree of multiple infection commonly met with (Sect. II, 1 (b)), much of which might prove to be of the direct kind; and also the facts as to yards-in-common, and as to infection between parents and infants; all suggest that the occurrence of a definite amount of direct personal infection in diarrhoea of the kind above described should receive due recognition.

(8) Finally, *the behaviour of the disease in the course of direct transmission is probably somewhat similar to that in typhoid fever: i.e.* where the fine splashings of stools are excluded, the striking distance is not great; it does not fly across air spaces but *tends rather to feel its way along*, chiefly perhaps on hands which have come into contact with the patient or with his stools or soiled napkins; or again, being taken in with food infected, in the course of its preparation, by hands or household utensils similarly polluted¹.

Its infective capabilities are of course largely held in check by the presence of large numbers of relatively immune persons. On the other hand, remarkable differences in infective virulence of different strains are found, just as in scarlet fever, which also, no doubt, in strains of low virulence, feels its way along in some such a manner as diarrhoea. In strains, however, of great virulence, both in the latter disease as well as in the former, the appearance of actual flying across air spaces is presented, so suddenly and directly is the infection transmitted. Such occurrences were noted in Bruce Low's instances, and also in many of the local foci and of the outbreaks in families in the two districts: the groundwork of the epidemic, however, was made up of a great many isolated cases, scattered between the clumps, and apparently almost devoid of infectious qualities.

¹ Such a moderate conception of direct communicability in diarrhoea is not at all incompatible with Vincent's experience, "that no infant has ever contracted the disease in the hospital" (Infants', Westminster); where the milk is described as specially protected "from the introduction of infectious matter from the wards," being brought in in separate bottles, stoppered till the moment of feeding. On the other hand, the conclusion deduced that "zymotic enteritis is in no sense of the word an infectious disease" does not receive any real support from such an observation. See references to Vincent (1910, pp. 8 and 9), and Sandilands (1910, p. 108), at the foot of this paper.

In conclusion, it might be stated that anything more than a reserved judgment is hardly warranted upon a question of such immense practical importance till a very considerable amount of evidence has been collected.

The above facts as to the manner of transmission are important from the standpoint of prevention; and in reference to them it might be mentioned that everything points to the probability that the amount of what has been classed as *direct infection within the home can be considerably reduced*, in both typhoid and diarrhoea, *by scrupulous care of the hands, and by extraordinary precautions as to isolating the patient, and by proper care in the handling of infection dejecta.*

(c) *Transmission by Fly carriers.*

The demonstration by laboratory experiments that flies can carry infective matter of diseases akin to diarrhoea can hardly be regarded as more than the merest preliminary to the determination of the responsibility of flies for the great seasonal outbursts of that disease. An attempt might be made to crystallize the complex issues there involved into the three following practical questions, categorical replies to which might fairly be demanded before the fly carrier theory can be regarded as completely established:

(1) *Do flies carry and communicate the infection of epidemic diarrhoea?*

(2) *Is any considerable part of the infection of the seasonal epidemic so carried?*

(3) *Are flies to be regarded as the active and necessary cause of the seasonal outburst?*

In putting the last question the influence of various passive factors, such as accumulation of susceptible persons and a certain amount of multiplication of infection in food, are for the moment set aside.

The second question is seen to embrace most considerations of really practical importance. The third is added to cover epidemiological issues. It is generally understood that satisfactory affirmative replies have not yet been given to the second and third questions.

The problem may be approached, in the first place, by means of fly-counts and subsequent comparison of the seasonal curves of fly prevalence and diarrhoea prevalence. Niven (1904-6, 1908-9) and Hamer (1908-10) provide data comprising eight sets of seasonal curves. The corre-

spondence of these pairs of curves—which was fairly good—is referred to at length at p. 727 *et seq.*

It is well to note that though by such curves correlation may be established between the two phenomena, it does not necessarily mean causation. They may be merely unrelated phenomena referable to a common causal factor, the temperature. Similarly, there is a large amount of evidence of other kinds, as that to be presently dealt with, accumulated around this question, not at all unfavourable to the theory of fly carriage, but not yielding sufficient warrant for the institution of wholesale preventive measures and the administrative outlay thereby entailed.

Confirmatory evidence therefore, of a directly experimental nature, is clearly demanded; and what appeared to the writer to be the three essential experiments may be outlined as follows:

(1) *A negative experiment*: that households living in houses rendered fly-proof by wire gauze or other means, though surrounded by infection on all sides, do not themselves develop the disease.

(2) *A positive experiment*: that households duly protected against all other chance of infection, and in which flies from infected houses have been liberated, develop abundant diarrhoea.

(3) *A bacteriological test*: that the specific organism can be demonstrated upon flies caught in diarrhoea houses, and also upon those flies which in the second experiment were held to have introduced infection into healthy households.

Though the third test may be at present inapplicable, the first and second should be quite practicable and present no greater difficulties than have been contended with in the case of Malaria and Yellow Fever; and confirmatory evidence yielded by them would give warrant enough for the institution of any practical measures to check the prevalence of flies or of diarrhoea.

The above suggestions are not included here for purely theoretical reasons: having become convinced of the necessity of such crucial tests the writer made some attempt to follow them up by making, during midsummer holidays in 1909, a personal test of the second experiment on two occasions, but without positive results. Batches of flies were caught in two houses in which several members were suffering from diarrhoea: these were allowed to inoculate food in a very thorough manner, but no attack resulted.

Many observations upon flies and fly-prevalence were made in the various districts; those bearing directly upon this enquiry are described

in detail in Section V, 4; and the conclusions, as to non-carriage of infection from the breeding ground and as to the frequent want of correspondence in different localities between numbers of flies and amount of diarrhoea, are there discussed.

From the immediately preceding sections (Sect. VII, 2 (a) and (b)) evidence can also be drawn in support of the fly theory.

The large amount of evidence for personal infection, except that supporting only the direct kind, is mostly evidence also for the possibility of fly carriage. Evidence as to clumping and radial spread from infective foci is specially suggestive, as well as those facts indicating that it is not the person so much as the house that provides the centre of infection. Thus, while not much evidence was collected as to the occupational or school infection, community of infection of neighbouring households was constantly noted, as if an individual was not so likely to prove a source of infection by direct transmission to those he mingles with, away from home, as to the neighbours adjacent to his house; within the precincts of which it must be noted his infectious dejecta are most likely to be deposited.

Some doubtful evidence as to houses facing south being more affected, and as to houses with rear premises facing one another not appearing thereby to be more liable to be mutually affected, might in each case be held to support fly carriage. Attention is specially directed to the complete tabulation of the evidence for fly carriage, as well as for case-to-case infection on p. 715 *et seq.* The subject of this section is also dealt with in Sects. V, 4 and VII, 3 (b) (i) (3).

(d) *Origin of infection from, or emanation out of, the Ground.*

In the preceding sections a strong case has been made out for personal origin of diarrhoea and for the continuity of case-to-case infection from season to season. It will be interesting to bring forward now certain facts which appear to point to a quite opposite conclusion, and which might be held to support a belief in origin of infection from the ground. In the writer's paper (1908), alluded to before, whilst reviewing the cause of the seasonal rise common to all infectious diseases, but expressly disclaiming any attempt to put forward new theories, a conservative effort was made to set forth all those facts which disfavour a too speedy desertion of those older beliefs, such as origin of infection from the soil, for the newer ones of fly carriage and continuity of personal infection.

Having become disencumbered, as regards the pre-epidemic period, of a necessary belief in the 4-ft. temperature theory in favour of the influence of a period (cf. pp. 686 and 733) of superficial temperature influences, the oft-suggested lodgment of the infection in the dust could come up for consideration; and it was suggested that the term "ground infection" should be substituted for "soil infection" so as to include infection from organisms lying *on* as well as *in* the ground. It was further suggested that infection might perhaps find its chief lodgment in the dust of the household, and that the seasonal rise of the disease was due simply to the maturing of a high degree of infection in such organisms during a period of certain accumulated temperatures, referred to here as the pre-epidemic period. A wider and more subtle conception of a ground origin is thereby provided, and one which is more proof against hostile attack; for the questions of moisture and of multiplication of organisms thereby lose any necessary bearing upon the question; and in view of the shelter, and of the very equable temperature provided within the household, the perplexing questions of maximum and minimum temperatures, and of the disturbing influences of rain and drought upon organisms growing out of doors in the soil, may also be set aside.

The chief argument adduced in support of a ground origin, apart from relations to meteorological and soil conditions of a general kind, was concerned with *the abrupt annual rise* of diarrhoea mortality, related always to the supervention of an extended period of certain definite temperature conditions (see Sect. VII, 3 (a), p. 718) external to the body. From the marked abruptness of the rise in diarrhoea, as well as from the apparent accompaniment, as in the seasonal rise of other diseases, of changes in infective and lethal virulence, it might be argued that the epidemic cases are not continuous with, and not always derived by multiplication of, the winter cases. Certain differences may apparently exist in the nature of summer and winter cases: thus, in the year 1905 for London, the percentage of deaths of infants under one year was relatively five times as great amongst the epidemic as amongst the winter cases. On the other hand, it may be perhaps that only a small proportion of the winter deaths represent specific cases out of which the epidemic cases can arise; the abruptness of junction of epidemic and inter-epidemic curves (see paper 1908, pp. 24 and 25) could be thus explained. Again, in many seasons, where the data are sufficiently massive, slight or minor rises can be seen leading up to the main rise of deaths; and it is possible that these might have been more often noted but for the very imperfect relation known to exist between deaths and cases; the latter

fact being held to be chiefly accountable for the very abrupt manner in which an epidemic will arise at the end of, say, a 10 weeks pre-epidemic period at the unfavourable temperature of 55° F. without any apparent signs of slight multiplication of cases leading up to it (cf. Paper 1908, Chart of Blackburn, p. 51). Collections of sickness data, *e.g.* those of Ballard and those from Mansfield (cf. Sect. VII, p. 722), suggest the gradual multiplication of winter into summer cases perhaps more strongly than the mortality data. Preceding spring and winter cases are discussed below.

Before passing on it may be remarked that the length of the pre-epidemic interval, but not of course the abruptness of the rise, might, as far as present data go, be quite acceptably explained by the fly carrier theory (cf. p. 733).

With regard to the Mansfield data, it should be noted that not only a sudden but *also a widespread appearance* of the disease occurred throughout the town shortly after the rise to summer temperatures. As a matter of fact a very special enquiry was undertaken with regard to these matters, and observations were made at carefully selected points, evenly distributed in every part of the borough; 15 of these localities were visited, comprising 4 to 20 houses in each. In nine of these areas cases were found to have already occurred during or immediately preceding the week ending June 20th. These nine areas were scattered all over the borough, there being four miles between the most extreme. Thirteen of the 15 localities had one or more cases occurring before June 27th and the remaining two developed cases within a few weeks afterwards. Thus early in the season, then, it was found possible, by house-to-house enquiries, to demonstrate the presence of cases of diarrhoea in every instance, before more than 20 houses had been visited in each case; in other words, the disease was found to have appeared simultaneously on all sides; and already it might be estimated that there were several hundreds of cases in the town. June 13th might be set down as the very earliest date of the commencement of the summer cases, although for about another week and a half the increase was hardly recognisable.

The important question now before us is to decide, *Whence do these ubiquitous foci of infection arise?*

The difficulty experienced, upon enquiring into the history of individual cases, in referring more than a small part of them to a personal source of infection, might be held to favour a ground rather than a personal origin; but other infectious diseases such as scarlet

fever, and diphtheria, present as great difficulties in this respect. Again, when the triangular and quadrilateral areas were separately examined, the epidemic was found to start more or less simultaneously in a score or more of isolated foci, scattered all over them, and apparently unrelated as regards their origin (cf. Charts I and II, App. : crosses are placed in the streets opposite houses containing such early cases). On a minute examination of the data as to 19, and 15, of these cases, commencing before July 3, and July 6, in the respective districts, it was found in the first place, that these cases were distributed amongst all ages, and to some extent in proportion to age and susceptibility. "Children" from 5 upwards were however represented in a smaller proportion. In the quadrilateral, "fathers" were curiously prominent; but in the triangle, young children in the second and third years were markedly in excess; and this appeared to be of special significance, as the 34 houses taken together were found to contain children at this age-period half as frequently again as the total attacked houses in the areas, while infants under one and other persons occurred in the usual proportions. Special attention must be called then to *the important part children in the second and third year play in the lighting up of the seasonal epidemic*. It might be presumed indeed that they are the chief means of carrying over infection from the preceding season. Firstly, because children under that age were practically all upon the breast at the end of the last epidemic, and therefore mostly unattacked. Secondly, because children of the second and third year are most subject to attack (cf. Table II), to chronic attacks (cf. Table XII), and to recurrence of attack (cf. Table XIV); and are therefore more likely than those at any other age to have had the disease in the preceding season, and to have retained infection or to have redeveloped an attack. Thirdly, cases of that age were more commonly met with than of other ages during the winter and spring preceding the epidemic of 1908. As regards the latter, histories of 11 cases were obtained of those occurring before June; five of these were of the above-mentioned age; one was under 1 year; and three, who were attacked in April, were fathers. From the latter occurrence, as well as from a consideration of their moderately high susceptibility and tendency to long and recurrent attacks (cf. Tables II, XII, XIV), the fact might again be insisted on that the disease we are dealing with is not an infantile disease, but one in which adults play an important part, in handing on infection as well as in other ways. The other fact must not be forgotten however that young children of the above-mentioned ages still play nevertheless

a more important part than adults. Again, persons subject to recurrent attacks were more than twice as numerous as usual. Finally, it could be easily assumed that certain recurrently affected babies were the means of lighting up the outbreak in their own neighbourhood; but, except possibly in a few instances, no certain connection of that kind could be traced, and the bulk of early cases appeared to spring up on all hands in an apparently wholly unconnected manner. It should be noted that the *main* rises in different districts, as will be discussed later, followed the appearance of these first scattered cases at widely different intervals (cf. the widely different dates of *main* rise in the triangle and quadrilateral); nevertheless the appearance of the substratum of scattered cases seemed practically simultaneous throughout the town.

The respective claims of the ground and personal infection theories are exhaustively treated on p. 715 *et seq.*, where the whole of the relevant facts are set out in tabular form. A few only need be specially noted here.

In the first place, the peculiar form of the typical diarrhoea curve, discussed at length on p. 723 *et seq.*, has been noted as affording evidence of case-to-case infection, from the regular manner of its rise. It is not improbable, however, that an epidemic, dependent wholly upon the maturation of infectivity in a ground organism, might present a curve of somewhat similar form. Variations of temperature in the different situations in which the organisms lie, and consequently in the date of completion of the process; and the increasing probabilities of infection with the passage of time; would sufficiently account for this result. As regards this possibility, in the spring of 1908, I made rough counts of the daisies as they appeared in the lawn, and found that in their case also the curve of increase was of regular form and apparently that of some *probability distribution*. Moreover, having reached their maximum prevalence, they appeared to decline while the summer temperatures were still rising from exhaustion of the function of florification; the latter however continuing, in some slight degree, until the autumn temperatures fell below a certain point. Thus, in all respects, a peculiarly close and interesting parallel with every detail of the explosive rise and subsequent exhaustion of the diarrhoea curve was presented.

Again, although infection may be always derived from a human source, being handed on by chronic cases through the winter, yet there is always the possibility of a revivification of virulence from symbiotic

influences exerted by non-specific organisms derived from the ground, which may gain entrance to the body at the beginning of summer; a lighting up of chronic cases or recurrent cases being thereby produced.

The major part of the evidence can be made to fit either theory; thus, the grouping of infected persons and houses, in point of time and place, may be assigned to infection from a common ground focus. All the facts favouring a personal origin are of course against a ground origin. Of the facts favouring a personal origin, one worthy of special note is the occurrence of cases in houses newly built upon clean meadow land (cf. p. 696). It is important to note (Table on p. 715 *et seq.*) that the ground theory is not supported by any other evidence than that of the abrupt rise of the mortality curve, a point which is however of rather doubtful interpretation.

Whatever value may be attached to the arguments of this section, the complete demonstration it affords of *the marked endemicity of diarrhoea* must remain as a fact of real practical importance. The truly epidemic diseases, such as small-pox with us, appear to have periods of total disappearance; subsequent epidemic prevalence being generally clearly traceable to spread from locality to locality. Diarrhoea, however, presents exactly opposite characteristics, being apparently the most markedly endemic of all the ordinary infectious diseases. Figuratively speaking, from the facts above presented, it might be said that the whole population of town and country is saturated with diarrhoea infection, which only awaits the appearance of the warm weather to become released on every side with epidemic violence.

(e) *Infection from Dust, Horse-manure, etc.*

Dust has been regarded as a source of infection, being in a manner a vehicle for carrying and depositing organisms, presumably mostly of a saprophytic type, or possibly a specific pathogenic variety, upon milk or food. Of course dust, using the term in a general sense, is too widely distributed in nature to account satisfactorily *per se* for the very particular epidemiological features and peculiar distribution of diarrhoea above set forth. The dust blows upon all houses and people alike, and moreover, in a dry spell of winter, may be more abundant than in an ordinary summer. Moreover, diarrhoea has been found by several observers to advance most rapidly in still weather; and thus there is very little evidence of dust being a more common vehicle of infection in this than in other infectious diseases.

Horse-manure, containing an organism, the *B. enteritidis sporogenes*, one of the organisms put forward as possibly the specific cause of diarrhoea, might be brought into contact with food as dust from the streets. Again, it is the commonest breeding ground of the fly; and the suggested connection of flies with diarrhoea also involves the possibility of carriage of infection, perhaps in the form of the particular bacillus above alluded to, or the *B. coli*, or some other organism, from collections of horse-manure, in which the fly most commonly breeds. With regard to this question several observations have been made that diarrhoea displays no greater prevalence around stables than elsewhere, and the writer's observations in 1908 also yield important evidence on this point, as already mentioned (cf. Sect. V, 4).

(f) *Milk Infection, Water Infection, Fruit and Food Infection, etc.*

The actual facts bearing on the possibility of infection by these means have already been dealt with under "Food" (Sect. VI). The possible epidemiological relations of diarrhoea to the bacterial content of food are dealt with later (cf. p. 746). It is only necessary to recall here the possibility that food might be infected either from a human or ground source; and by both the direct method, as by dust from the ground or by human contact, and by the indirect method of fly infection.

(g) *Conclusions: the evidence for Personal Infection, Ground Infection, Direct Personal Infection, and Fly Carriage.*

PERSONAL INFECTION VERSUS GROUND INFECTION.

A.—*Evidence supporting a Personal Origin only.*

- (1) The evidence of yards-in-common (cf. p. 689 *et seq.*).
- (2) Possible perambulation of a locality (cf. p. 695).
- (3) Possible movement from one large district to another (cf. p. 695).
Irregular evolution of epidemic in different districts (cf. p. 737).
Irregular development, in point of time, of time and place clumps (cf. p. 688).
- (4) Persons traced carrying infection from other parts of the town (cf. p. 696).
- (5) Excessive incidence, particularly upon parents, within houses containing infants (cf. Table V).

- (6) The mass influence of collections of houses containing infants (cf. p. 651).
- (7) Spread to houses newly built (cf. p. 696).
- (8) The common occurrence of winter cases (cf. p. 697).
- (9) The numerous instances met with, pointing unmistakably to direct personal infection (cf. Sect. VII, 2 (b)).
- (10) Considerations as to frequent faecal pollution of the household (cf. p. 660 *et seq.*).
- (11) The regular form of the rising curve, suggesting case-to-case increase, in geometrical ratio (cf. p. 723 *et seq.*).
- (12) Solitary cases generally precede for some time the outbreaks in the clumps (cf. p. 695).
- (13) The large amount of evidence supporting the fly theory at the same time supports a personal origin of infection (cf. p. 717).
- (14) The absence of any proof *against* a personal origin of infection.

B.—Evidence not unfavourable to either Theory, but perhaps favouring a Personal Origin.

- (1) Multiple cases in the family (cf. p. 611).
 - (2) Associated persons and houses seldom attacked together, but generally one after the other (cf. p. 694).
 - (3) The regular form of the rising curve, suggesting case-to-case increase, in geometrical ratio (cf. p. 723 *et seq.*).
 - (4) The presence of possible connecting cases through the winter, and the large proportion of persons chronically and recurrently affected at the beginning of the season (cf. p. 712).
 - (5) The much higher incidence upon the members of attacked households in the high incidence clumps or sections, than in the low incidence sections (cf. Table XXV).
 - (6) The smothering of the differential influence of dirtiness in the clumps (cf. p. 646 *et seq.*).
 - (7) Excessive incidence within dirty families (cf. p. 651 *et seq.*).
 - (8) The general influence of dirtiness in increasing the liability of houses to attack (cf. Sect. V, 1).
 - (9)* No excessive incidence around stables: this suggests that flies obtain infection from a personal source (cf. Sect. V, 4).
 - (10)* Variations of prevalence with variations of temperature (cf. p. 722 *et seq.* and p. 739 *et seq.*).
- * ((9) and (10) are contingent upon the fly theory being proved.)

C.—Evidence not unfavourable to either Theory.

(1) Clumping of houses in point of place (cf. p. 688 *et seq.* and Chart VII).

(2) Clumping of houses in point of time (cf. p. 688 *et seq.* and Chart VII).

(3) Clumping of persons, *i.e.* multiple cases in the family (cf. p. 611).

(4) The important part played by the household as a whole; suggesting that the house, and not the person, is the centre of infection. (cf. p. 709).

D.—Evidence not unfavourable to either Theory, but perhaps favouring a Ground Origin.

(1) The apparently simultaneous and wide-spread appearance of infection at the beginning of the season (cf. Sect. VII, 2 (*d*)).

(2)* The pre-epidemic interval of certain accumulated temperature influences, without the body (cf. Sect. VII, 3 (*a*)).

(3)* The very low level of winter cases (cf. Chart B, p. 720).

* ((2) and (3) are contingent upon the fly theory remaining unproved.)

E.—Evidence supporting a Ground Origin only.

(1) The abrupt rise of the epidemic mortality curve from the inter-epidemic curve (cf. Sect. VII, 2 (*d*)).

N.B.—Where, in the above, the classification is doubtful, inclusion of the same item of evidence in two groups has sometimes been resorted to.

FLY CARRIAGE AND DIRECT PERSONAL INFECTION.

A.—Evidence specially favouring Fly Carriage.

(1) The low level of the winter cases—in the absence of flies (cf. Chart B, p. 720).

(2) The facts suggesting that the house, and not the individual, is the centre of infection (cf. p. 709).

(3) The sudden outbreak in a clump, supervening upon solitary preceding cases, suggests that flies have suddenly gained access to infection (cf. p. 695).

(4) The fact that within the clump, infection appears to some extent to rain down equally upon all persons and houses included, suggests systematic dissemination by flies (cf. p. 655 and p. 646 *et seq.*).

(5) Variations of prevalence with variations of temperature (cf. p. 722 *et seq.* and p. 739 *et seq.*).

(6) The almost identical temperature limitations of fly and diarrhoea prevalences, as regards their rise and fall: the significant immobility of the diarrhoea curve till the first fortnight of favourable fly temperatures has passed by: the parallel with cholera (cf. p. 727 *et seq.*).

(7) The correlation of the fly and diarrhoea curves is such as to be quite compatible with a theory of causal connection between flies and diarrhoea (cf. p. 727 *et seq.*).

(8) The large amount of evidence for a personal origin of infection, excepting some mentioned below, is mostly evidence also for fly carriage (see above).

(9) No real evidence has been produced *against* the fly theory.

B.—Evidence specially favouring Direct Personal Infection.

(1) Continued occurrence of cases during the winter time, when flies are absent: the low level maintained is perhaps against direct methods playing a large part in infection (cf. Chart B, p. 720, and p. 701).

(2) Instances where it seems almost absurd to entertain the possibility of any other mode of infection (cf. Sect. VII, 2 (b)).

(3) Instances of infection from babies to parents, and *vice versa* (cf. p. 701).

(4) Considerations as to frequent faecal pollution of the household (cf. p. 660).

(5) The evidence of yards-in-common (cf. p. 689 *et seq.*).

(6) Most of the evidence for a personal origin of infection (see above) might be held to be not unfavourable to direct personal infection.

3. *Some Factors Governing Epidemic Prevalence of Diarrhoea.*

(a) *Influences determining the Rise of the Seasonal Epidemic Curve.*

(i) *Temperature and the rise of Diarrhoea Mortality.*

The relations of epidemic diarrhoea to the temperature were dealt with at some length in my paper "Season and Disease, etc." (1908), but almost wholly as regards data derived from the mortality returns.

It was found for Nottingham, London, and most large cities of the United Kingdom, that after the arrival, and continuance, without intermission, of the mean air temperature at 60° F. for a period of four or five weeks, the *main* seasonal rise of diarrhoea mortality took place. The last fortnight or ten days of the period corresponds to the average duration of fatal attacks; leaving an interval of about three weeks before the main series of fatal attacks commence. At lower temperatures, or when remissions occur, this interval is proportionately extended until the required sum of accumulated temperatures is complete. Thus, at the relatively very unfavourable temperature of 55° F., below which only a very slight and rapidly diminishing influence appears to be exerted, the whole period may occupy as much as ten weeks.

On the other hand the potency of temperatures appears to increase for some distance above 60° F.

The tendency to abruptness of rise of the mortality from the winter level has already been discussed (cf. Sect. VII, 2 (*d*)).

A general conclusion from the examination of several hundred charts is that, upon a rise to favourable mean air temperatures of 60° F., or a little below, there is: firstly, an interval of two weeks, during which no increase of diarrhoea cases occurs, or four weeks in the case of deaths: afterwards, a slight rise occurs; but an additional period of one to two weeks, or a little more, may elapse before what has been alluded to as the *main* rise occurs (cf. Chart B, p. 720). The period extending from the arrival at temperatures exerting any influence at all upon the rise of diarrhoea, up to the moment of the *main* rise, is referred to in this and the former paper as the "pre-epidemic" period.

In proceeding to the examination of the mortality curve, it is important to note the very strict limitations of the mortality data of diarrhoea, as a gauge, or as a reliable representation in miniature, of the course of the total prevalence of all cases of the disease; particularly as regards comparisons of different towns. Firstly, the deaths comprise only a very small fraction of all cases and therefore may chance to fall very irregularly with relation to the curve of all cases. Secondly, they are limited in greater part to infants under one year. Thirdly, in deaths of the latter class, moreover, a fatal termination is largely if not mostly decided by circumstances of previous health and social environment: and fourthly, these last-mentioned factors differ markedly in different towns. For these and other reasons, the principles to be laid down in the present and following sections, as to the effect of temperature upon the mortality curve, cannot be suitably demonstrated except in towns

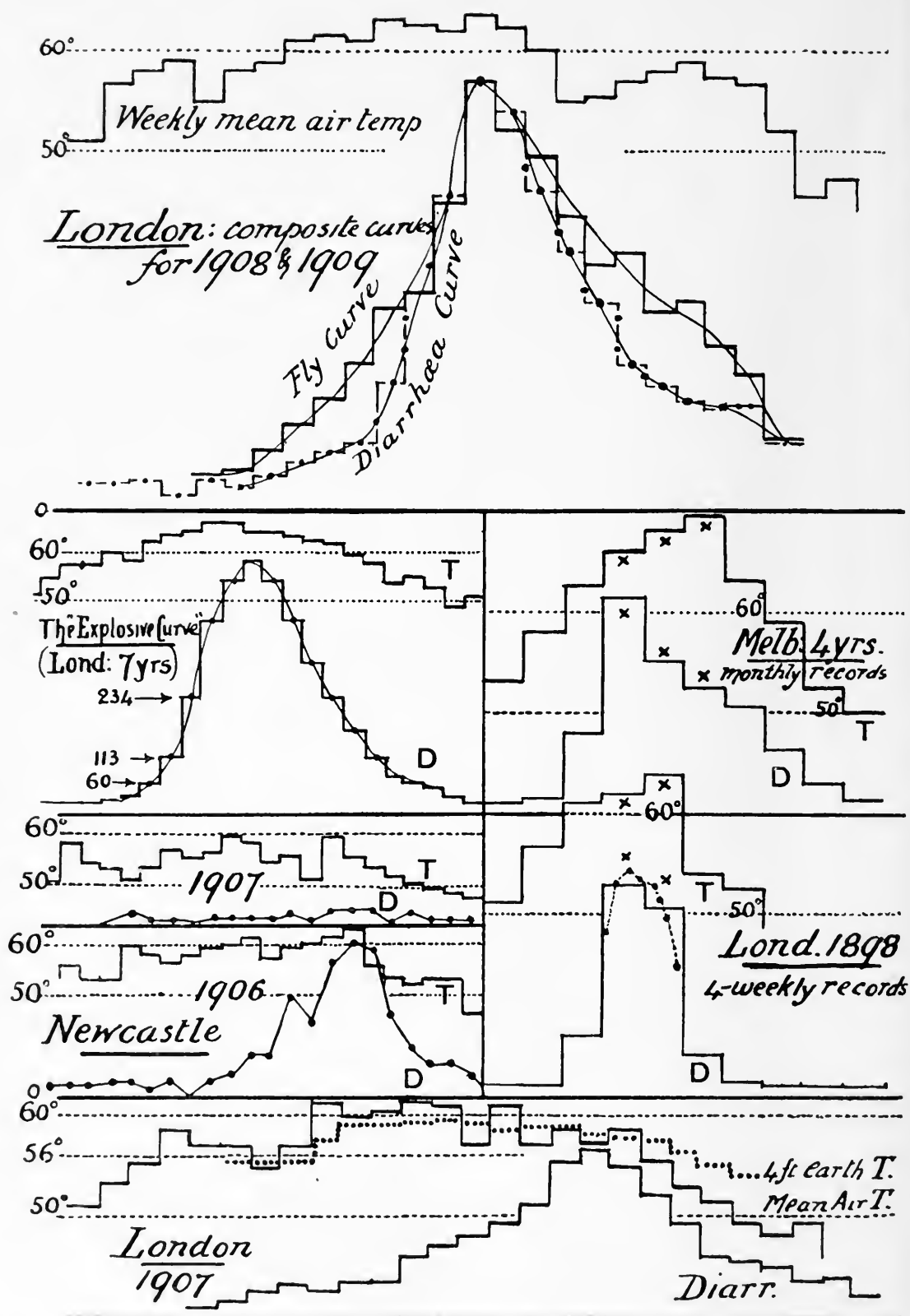


CHART B. Illustrating the typically explosive form of the diarrhoea epidemic curve (Lond. 7 years); epidemic decline in spite of a continuing high temperature (Melb. and Lond. 1898); the inhibition of the epidemic rise by unfavourable temperatures (Newcastle); various peculiar relations to air and 4-ft. temperatures in irregular seasons (Lond. 1907); the inter-relations of the curves of fly and diarrhoea prevalence (Lond. 1908 and 1909), etc. (See Explanatory Notes on p. 721.)

CHART B.—*Explanatory Notes*.

N.B.—Weekly records are used throughout the charts, except where otherwise stated.

The Diarrhoea records (D.) are all Mortality data (Registrar-General), but all being moved two week-intervals to the left, they represent Onsets of Fatal Attacks. Mean Air Temperatures (T.) are used throughout (Greenwich for London).

London: composite curves of temp., diarrhoea, and fly prevalence, for 1908 and 1909 (e.g. the week at summit represents the sum or average of the weeks ending August 15 and 14, in the respective years). The weekly records of fly prevalence are used by kind permission of Dr Hamer (1908-10). In drawing the thin line a little free, but quite legitimate, smoothing of the fly curve has been practised. The scale of the two curves is so arranged that the apices of the fly and diarrhoea curves coincide: the amount of divergence of the slopes of the two curves from one another provides then to some extent *a graphic representation of the amount of difference in the respective methods of increase, or decrease, of flies and of diarrhoea cases.*

The "Explosive Curve": London, seven years' composite curves of temp. and diarrhoea (1897-1906, less the three irregular years 1900, 1902, and 1903). The curve is of the graceful and very regular explosive form, and the increase of cases in geometrical ratio is illustrated in the first part of the ascent where the total of deaths is approximately doubled from week to week.

Melbourne: four years' composite curves of temp. and diarrhoea mortality (1894-8), Dr R. R. Stawell (1899): and *London, 1898.* Monthly or four-weekly records are used. These charts show epidemic exhaustion occurring respectively two months and one month before the decline of temperature: the rounded explosive summit of the London curve and its subsequent excessively abrupt fall, both bear testimony to the fact that epidemic exhaustion was becoming decidedly marked in degree. Note in both countries that the same period, two months, was taken in reaching the acme; also the similar temperature limitations.

Newcastle, 1907 and 1906. Showing the power to completely inhibit epidemic rise possessed by unfavourable temperatures. The diarrhoea curve for 1907 became almost a straight line; on the other hand that for 1906, drawn to the same scale, showed that there were local possibilities of a huge epidemic rise when temperatures were favourable. On two occasions in 1907 the pre-epidemic conditions appeared to be almost fulfilled when the supervention of a lengthy and continuous fall of temperature inhibited epidemic increase.

London, 1907. Note, in this irregular year, the failure of the 56° 4 ft. temperature rule; the rise of cases against a falling temperature; the relatively meagre proportions of the epidemic (less than half the average number of cases) owing to rise from a low temperature-plateau; the tailing out of the curve with the lingering of the temperature for some weeks just below 50° F.

Throughout the above charts the temperature conditions of the pre-epidemic period are well illustrated, as also the limiting influence of temperatures at or about 50° F. No very good examples of temperature notching of the diarrhoea curve are however included. A much more complete selection of charts is available in the writer's previous paper (1908).

with populations well over 100,000 ; in fact the effects upon prevalence, and the inherent qualities of the curve discussed later, only constantly emerge in massive statistical data, such as London alone presents.

(ii) *Temperature, and the rise of Diarrhoea Sickness.*

Unfortunately data as to non-fatal as well as fatal cases of diarrhoea are still very scarce. Those that are available however appear to bear out the above conclusions as to the lapse of the equivalent of about a fortnight at 60° F., of the air temperature, before any epidemic cases of sickness begin to occur. A comparison of Ballard's (1887-8, Chart V) returns of diarrhoea sickness, taken from the Poor Law Practice at Islington for the ten years 1857-66, with the temperature at Greenwich, supports this conclusion ; as also the sickness data obtained at Mansfield. Chart IV, App., sets out the latter data. It shows that the temperature, after remaining at 60° F. for only a week, fell considerably, and the pre-epidemic period was somewhat lengthened thereby ; the rise occurred in strict accordance, however, with the rules above laid down (cf. also Sect. VII, 2 (d)).

(b) *Influences determining the Precise Outline of the Seasonal Epidemic Curve.*

(i) *Firstly, as regards the Mortality Curve.*

(1) *Temperature: limiting the extent of the epidemic mortality curve, and producing irregularities in its outline.*

These matters were also enquired into in the paper (1908) above referred to ; although it is regretted that, owing to pressure of space, the reasoned demonstration from the charts was so prejudicially curtailed. It is practically essential to the full appreciation of this enquiry into the phenomena of the epidemic curve, that the details should be carefully followed out upon those charts, only a few of which (cf. Chart B, p. 720) could be reproduced in the present paper. Reasons have already been given (p. 719) why not too much must be expected of the mortality curve in these matters. In addition, in the present instance, the irregular evolution in different districts must be considered : thus a temperature notch may happen to be filled up by a large local explosion (cf. p. 737 *et seq.*) when the data are not massive.

Briefly, *the influence of temperature upon the seasonal epidemicity of diarrhoea is, in a sense, an all-powerful one ; i.e. to the extent—as far as conditions in this country are concerned—that, firstly, it is*

absolutely necessary to the very appearance of the epidemic; failure of the summer temperatures to reach a certain height for a certain time will inhibit its appearance altogether, as not infrequently happens in elevated or northerly towns (cf. the season of 1907, in Edinburgh, Glasgow, and Newcastle (Chart B), with other years). Secondly, it unfailingly brings the epidemic mortality to a standstill a fortnight after the weekly air temperatures have passed, in a decided way, below 50° F. Thirdly, between these two terminal points of the mortality curve it keeps a tight hold of the epidemic prevalence, producing notchings ten days to a fortnight after the temperature variations. Its grip is in fact always apparent; although in accordance with the explosive nature of the curve, the latter rises sometimes with a falling temperature, and so appears to contradict the above statement. In such a case, careful measurement will always show that temperature, as a constant depressing force, has really had a definite effect by slowing down the velocity of the rise. A rise was never found to occur against the falling temperature, when in the neighbourhood of 50° F.

None of the above rules as to temperature were more strictly conformed to than that of the complete fall to winter level when the temperature passed below 50° F. In examining some hundreds of seasonal curves in about 20 of the largest towns of the three kingdoms, no exceptions, but one of a doubtful nature, were found to this rule, however early the rise of the epidemic occurred. The interesting and just perceptible tailing out of the epidemic frequently observed when the average weekly air temperature continued for several weeks at 50° F. or a little below, before falling decidedly, shows the tendency for some slight prevalence to continue as long as the temperature just remains above, or at, this point; e.g. London and Sheffield, 1907; and London, Liverpool, Birmingham and Glasgow, 1904. Above these limiting temperatures, however, with every degree of rise, there was found to be correspondingly greater potency in producing increase of prevalence.

- (2) *Gradual Exhaustion of Epidemic Potential as the epidemic progresses: determining the typical explosive form of the mortality curve, and gradually reducing the degree of the reaction to temperature.*

The typically explosive form of the epidemic curve, very typical of an infectious disease (cf. also p. 713), has been carefully studied by Brownlee (1905-6) and others. It is, in effect, a graphic representation of the

resultant between two opposing factors: firstly, the tendency to indefinitely continued multiplication of cases, which appears to proceed by regular geometrical progression (cf. Chart B, p. 720, Lond. 7 years); and secondly, an influence directly retarding this process, and acting throughout the epidemic so as to eventually bring about its complete cessation, at a rate, according to the authority named above, approaching to the terms of a geometrical progression. The question whether this retarding influence is dependent upon decrease in infective virulence of the causative organism or in the amount of susceptibility of the population, can be avoided by alluding to it as exhaustion of "epidemic potential," the latter term referring to the head of power for epidemic spread, present at any particular stage of the epidemic, as gauged by the highness of the infectivity over the lowness of the insusceptibility of the population. The resulting curve then, when not distorted by irregularities of temperature, has the well-known and characteristically regular form, with something of a rocket-like ascent, slowly and gracefully arching over to perhaps, though not necessarily, a rather more gradual fall (cf. Chart B, "The Explosive Curve").

The progressive exhaustion of epidemic potential introduces an influence qualifying, but be it noted, in no sense disproving, the laws as to the effects of temperature above laid down. The movements of the curve are in fact completely dependent upon the precise inter-relation, at any given moment, of the two interacting factors: temperature, and degree of epidemic potential. It is obvious, from inspection of a typically explosive curve, that up to the moment the acme is reached, epidemic potential is a positive influence, producing increase in cases; after that point is passed, however, it is a negative one, producing a constant decrease. If then, on the up-slope of the curve, the temperature should be rising, an exaggerated effect upon the increase of prevalence would be produced, from the fact that the two separate influences are pulling together: whereas an exactly equal rise of temperature after the acme is passed would produce a much smaller effect, from the fact that the two influences are here in opposition. Conversely, a fall of temperature would produce the greatest visible effect upon the down-slope, and show the least result in diminution of cases upon the up-slope.

With this recognition of the two interacting factors, quite an interesting set of apparent anomalies of reactions to temperature are at once elucidated. Thus, the fact of a late diarrhoea epidemic forcing its way up for a few weeks after a moderate fall from summer temperatures

has set in, will be seen, by applying the above principles, to be in no way incompatible with the constant controlling influence of temperature upon diarrhoea prevalence (cf. London, 1907 (Chart B), 1902, 1903 and 1905). Again, it is evident that if a rise to certain favourable summer temperatures should take place at the beginning of the season, and if these temperatures are thereafter maintained indefinitely at a constant level, the diarrhoea prevalence will not also be indefinitely maintained at a certain fixed level; but, as a matter of fact, the fixing of the temperature at a constant level merely serves to exclude the latter as a disturbing factor—exhaustion of epidemic potential being given full play to reveal itself in a perfect curve of typical explosive outline. Here, the influence of temperature, which becomes constant, would be of course responsible; firstly, for the fact that there should have been a rise at all; and secondly, for the highness of the point to which the epidemic curve rose, *i.e.* for the highness of the acme. However, having been “drawn up,” so to speak, to that highest possible point of prevalence, the curve would at once commence to descend in a regular manner, but always tending to indefinitely postpone the final and complete fall to winter level for as long as the air temperature maintained itself above the 50° F. limit. Thus besides rising against a falling temperature, the diarrhoea curve may also fall away from a maintained or even slightly rising temperature; both phenomena being still however consistent with a constant controlling temperature influence. Cf. London, 1898 (Chart B), 1899, 1895 and 1906, where these facts are most interestingly illustrated, and considerable falls in prevalence occur, during four or five weeks of high temperatures continuing at about 65° F., or even rising slowly to as high as 70° F.

Many of the London seasons were, however, so short as to give no opportunity of showing epidemic exhaustion; there can however be no doubt of its occurrence in such prolonged hot summers as those just mentioned. To establish however the matter altogether beyond question, a reference may be made to warmer countries having a much longer summer time, where a marked decline of the diarrhoea may commence as long as two months before the summer temperatures have reached their maximum (cf. Chart B, p. 720, Melb.). The most curious fact of the phenomenon is, that in different countries—in large cities at any rate—there is a tendency, probably depending to some extent upon a similar concentration of susceptible material in affected districts, for the time taken in the ascent to the maximum to be about the same in all cases, altogether without reference to the duration of

the summer. Thus in the London charts the ascent averaged about seven weeks (cf. Chart B, Lond. 7 years and 1898); while in Melbourne it was again something under eight weeks (cf. Chart B, Melb.), although, owing to the longer summer, the epidemic in the latter instance lasted for 30 weeks, *i.e.* twice as long as in London; the sudden uprush and rapid falling away from the acme, as if the epidemic had over-reached itself, is thus very marked; this being followed by a more leisurely fall, although the temperature is still rising for two months or more, and the winter level is not reached till 50° F. has been passed, just as in London.

Again, from the fact that the greatest velocity of the curve is found on the two slopes just below the summit; and the least, at the summit and towards the base; notchings due to sudden alterations in temperature are graphically most apparent in the latter positions; very great alterations of temperature being required to produce notchings upon the slopes. The frequently observed sudden falling away, when the acme is passed, is also explained by the above fact of the very great velocity of the curve at that particular point, and also by the fact that, in the data presented, there was generally a falling temperature at that time helping to accelerate the descent.

Finally, it also follows, that the greatness of the height to which the curve rises, *i.e.* the maximum weekly prevalence attained, is proportionate, other things being equal, to the general height of the temperature plateau from which it rises. Thus, the tallest curve in a series of seasons, if other factors were not unequal, should be the one where there was not only a very high temperature during the week of maximum prevalence, but where also there had been generally high temperatures for some time preceding, leading up—uninterruptedly—to the week of the acme. Cf. London, 1904 and 1897, which bear this out. From these and other considerations it is apparent why comparisons of epidemic prevalence with the average temperature of the third quarter do not give very regular results: additional information as to the highest temperature attained would help the comparison. Again, in cold seasons, where the epidemic is abnormally late in appearance, it practically always happens that the rise when it occurs is from a comparatively low plateau of temperature. Hence the almost invariable absence of tallness in these curves. While from the fact that the fall to 50° F. seldom takes place much later than in other seasons, it happens that they are also short in length, *i.e.* in duration (cf. Chart B, London, 1907). Such epidemics, rising from low temperature levels, are therefore

almost invariably of insignificant proportions. Yet, from the deferred date of onset, it might be contended that the susceptibility of the population, and the consequent degree of epidemic potential, would be even greater than in the warm early season, so that the epidemic might be expected to exhibit great expansive power and to continue to exist beyond the temperature limits observed in other seasons. The fact that it certainly does not do this therefore provides a demonstration, in another way, that *the explosive power of an epidemic, as well as the amount of prevalence, depends for its existence upon, and varies directly with, the height of the accompanying temperature.*

Brownlee (1905-6), in testing the fit of epidemic curves to various theoretical distributions, found that in composite curves of diarrhoea for long series of years in London and Glasgow, a good fit could not be obtained at the apices. This is evidently due to the disturbing influence of changes of temperature upon the normal evolution of the explosive curve. Thus, the temperature during the ascent of the curve, is generally rising; the rate of rise is therefore being continually increased, and the apex of the curve is drawn up higher, with a steeper up-slope, than it would otherwise have been had the temperature remained constant. Again, since the apices of the composite temperature and diarrhoea curves will probably not coincide (cf. Chart B, p. 720, Lond. seven years' curve)—the temperature tending to fall a little earlier or later than the diarrhoea—the apex of the latter curve will be accordingly asymmetrical; being pulled over to one side or the other.

(3) *The suggested inter-relation of Temperature, Flies, and Diarrhoea.*

Of the all-importance of temperature in this epidemic disease there can be no kind of doubt; and it was well to follow out its relations to diarrhoea prevalence to complete finality before introducing the discussion of fly carriage and other indirect means through which it is suggested the effects of temperature are produced.

The suggested influence of fly carriage must now be considered at some length, so as to determine what part of, or whether the whole of, the phenomena of the epidemic and mortality sickness curves, above noted, might be referable to and can be completely explained by this factor, including of course both the varying effects of temperature, and the phenomena of epidemic exhaustion. Curves of fly prevalence are now available for comparison with the diarrhoea mortality curve for five years at Manchester (Niven: 1904-6, 1908-9), and three years at London

(Hamer: 1908-10); and it will be interesting, and somewhat necessary, to compare the above observed relations of temperature and diarrhoea with a possible third relation to fly prevalence, from the evidence collected by these observers. There is on casual inspection a general correspondence in the general form, and in the notchings, of the fly and diarrhoea curves for any one year; but this might be attributed simply to both being dependent upon a common influence, the temperature; without there being necessarily any real causal relationship between them themselves. If the seasonal prevalence of flies determines *all* the phenomena of the epidemic curve, then by far the most reasonable conclusion to be deduced from this would be that the diarrhoea curve should be found to follow *exactly* the outline of the fly curve. *This it does not do in any* of the eight separate seasons above mentioned. The diarrhoea curve varies from the fly curve chiefly in two respects—

(1) Firstly, *the response of the diarrhoea curve to the rise of the fly curve is a very tardy one, and the rate of rise is different.* Some slight rise is generally evident about the time of the earliest increase in fly prevalence; but in three of the seasons (London 1908, 1909, and Manchester 1906) periods of three weeks elapsed before any considerable response of the diarrhoea curve was visible, or five weeks before the main rise in deaths took place (cf. Chart B, p. 720, Lond. 1908-9). Several of the records do not show the early and late relations of the two curves. This lagging behind the rise of fly prevalence is very similar then to the lagging of the diarrhoea behind the temperature, on its first rise to summer level, as discussed in a preceding section (p. 722 *et seq.*). For explanation of this occurrence there seems to be no need to go further than suppose that, whatever the means by which the high degree of temperature, or the fly prevalence, “draws up” the curve, the utmost degree of prevalence they have power to induce cannot be induced forthwith, but has to be patiently evolved by multiplication from an amount of infection of relatively microscopic proportions; this multiplication being in the nature of a geometrical progression, as suggested by the usual rate of increase as the main rise becomes definitely established (cf. Chart B, Lond. 7 yrs.). When once, however, the terms of this progression have reached an appreciable size, the charted curve shows that apparently sudden upward rush so characteristic of a graphic representation of this mode of multiplication, and the rate of increase of diarrhoea soon outstrips the rate of increase of flies: for the increase of flies more closely approaches an ordinary arithmetical progression, each of the slopes of the fly curve tending, to some extent, to approximate to a

straight line (cf. Chart B, Lond. Composite curves, 1908-9). Since however the temperature is not generally constant during the ascent of the fly curve, but is usually rising a little, it follows that there may be irregular acceleration of this arithmetical rate of increase, with consequent curving inwards of the upward slope. The lagging behind of the diarrhoea, and subsequent outstripping of the fly increase, produces a curving or sagging inward of the diarrhoea up-slope, away from the up-slope of the fly curve; an appearance which is actually observed or suggested in *every one* of the eight seasonal records provided (cf. Chart. B), and there can therefore be no doubt as to the reality of its occurrence.

In accepting the above view of the matter, we are furnished with a second and most important demonstration (cf. Sect. V, 4, p. 665) of the fact that the infection of the disease does not arrive on the bodies of the flies from the manure or refuse heaps in which they are bred: in which case the up-slope of the diarrhoea curve would follow quite closely the course of the fly curve: on the contrary, the rise of the diarrhoea curve, by geometrical progression, almost certainly relates to the origin and multiplication of infection by transmission from person to person.

(2) Secondly, *the rate of fall also of the diarrhoea curve is different*, the down-slope sagging inwards away from the fly curve, as happened also on the up-slope. There can be no doubt again as to the occurrence of this difference; it is also observed, or suggested, in *every one* of the eight records (cf. Chart. B, London curves, 1908 and 1909); and thus it is evident that the phenomenon of epidemic exhaustion, at least in part, could not be explained by exhaustion of the function of multiplication of flies in protracted summers, or by any cause, such as disease of the flies themselves, which might produce the fall in fly prevalence. At the period of maximum prevalence the dissemination of infective matter throughout the population, is also at its maximum; the diarrhoea, therefore, if there were no such deterrent as exhaustion of susceptible persons or of the infectivity of the causative organism, should thereafter necessarily follow closely, if not actually pass above, the outline of the fly curve; and only decrease when forced down by, and in close company with, the decline of fly prevalence. The fact that, after passing the acme, it fell away from the fly curve, necessarily proves the presence of some causative factor, apart from fly prevalence; and the rapid fall away from the acme, so characteristic of the decline of the typical explosive curve, before alluded to, at once points to intrinsic exhaustion of epidemic

potential as the factor in question¹. In addition, however, there is quite possibly some exhaustion or falling away of the fly prevalence from the temperature in protracted hot summers; as it is difficult not to believe that the function of fly multiplication has also natural limitations to its seasonal rage, notwithstanding the maintenance of favourable summer temperatures: as in the case of exhaustion of florification of early flowering plants. There is a slight suggestion of this in one season only. Data collected in warm countries having longer summers, where epidemic exhaustion is exhibited weeks and months before the mid-summer temperature is reached, should give interesting information on this point; or even London data, if fly counts could be made in such a protracted summer as 1899. The undoubted existence of intrinsic exhaustion in diarrhoea makes it plain that indefinitely continued multiplication of flies will not be accompanied by a similar indefinite increase in diarrhoea cases: at the lower levels of prevalence there is possibly, however, a fairly definite relation between the numbers of flies and the numbers of cases.

The continued rise of diarrhoea after the decline of summer temperatures has set in has been noted as a phenomenon of the epidemic curve not inconsistent with a strong controlling influence of temperature. A similar continued rise against temperature for two or three weeks is observable in the fly curve; closely accompanying, and afterwards declining with, the diarrhoea curve; in 1907 and 1908 at London, and in 1905 and 1908 at Manchester. As in the case of diarrhoea, the strong influence of temperature is invariably evidenced in a rapid decrease in the rate of multiplication; but in the above examples, from the fact that the temperature did not fall to sufficiently unfavourable levels, the decrease in the rate was not ocularly demonstrated upon the chart as an actual fall of the curve, as it was not sufficient to produce at once a smaller number of flies in the weeks immediately following the fall of temperature.

This occurrence has important bearing upon the question as to whether other factors, including the degree of *activity of flies*, upon which temperature may have some effect, are as important as the relative number of flies present. The writer (1908, p. 37) was led to suggest the probable importance of the former factor from noticing the

¹ The falling away of the diarrhoea curve, and the possible explanation of exhaustion of susceptible persons, has been mentioned by Niven (1910). The fact was noted here, however, and Chart B, Lond. 1908-9, was constructed, before acquaintance was made with that paper. See reference at foot of this paper.

marked precipitation of flies upon doors and window-frames upon sudden falls of temperature to somewhere below 54° F.; a phenomenon which, further, may afford a reasonable explanation of the curious fact of the almost immediate drop in the numbers of flies caught and of cases of diarrhoea occurring with a fall of temperature. In the above examples of seasons, however, the fall of temperature must have decreased the activity of flies; the fact that the increase of diarrhoea, notwithstanding, continued as long as the increase in flies also continued, showed that the diarrhoea varied with the numbers of flies rather than with the amount of movement of the flies: the latter factor thus apparently being, at most, one of only subordinate importance: all this of course being subject to ultimate proof of the fly carrier theory. It might again be suggested that the higher rate of increase of diarrhoea than of fly prevalence, in approaching the acme, is due to the increased activity of flies at the higher temperatures which generally prevail about that time; increasing thereby the infective potentialities of the flies. But this objection cannot be seriously entertained: for, apart from any slight bearing upon the matter of the observation as to the rise against temperature made above, it is also obvious from most of the prevalence data that a definite difference exists in the rate of rise of the two curves, whatever be the movement of the temperature. Thus, in Chart B, Lond. 1908-9, during the three weeks preceding the week of maximum diarrhoea prevalence—which were those in which the whole of the outstripping of the fly curve took place—there was actually a slightly falling temperature.

All the foregoing, as to the inter-relations of diarrhoea, fly prevalence, and temperature, is essentially contained in the following three facts (cf. Chart B, Lond. 1908-9):

(1) *All three curves are definitely correlated one to the other; but the fly curve is more closely correlated to the temperature curve than the diarrhoea curve is, getting more rapidly into relation with it at the early part of the season and appearing more loath to fall away from it later on; its behaviour being apparently quite compatible with the theory that fly prevalence is an intermediary factor between temperature and diarrhoea.*

(2) *The fly curve, however, does not render an absolutely mechanical obedience to its controlling influence—the temperature; but shows great independence of movement, dependent upon intrinsic factors, concerned, amongst other things, with the exigencies of fly breeding. Thus, it both lags behind the temperature in rising, owing to the time taken in the*

multiplying process; and shows small response to rises of temperature on the falling curve, from the fly prevalence being largely subject to the egg-laying of several weeks before. Both these features were also present in diarrhoea, but in a somewhat magnified degree for reasons next to be discussed.

(3) *In the same way*, it is important to note, *the diarrhoea curve also shows some independence of movement of*, what may prove to be its controlling factor, *the fly prevalence*; owing to the intrusion of certain intrinsic factors: lagging behind the rising fly curve, from the inertia of case-to-case multiplication; and falling away from it later on from exhaustion of infection or of the material to infect.

An explanation is afforded by the latter facts, and by others formerly given, why, if the numbers of flies become stationary on the ascending curve, the diarrhoea may continue to rise; while, if occurring on the descending slope, the diarrhoea curve may continue to fall, sometimes also appearing to fall away before the acme of fly prevalence is reached. A similar relation of diarrhoea has already been noted with regard to a stationary temperature (cf. p. 725). Again, just as with regard to temperature, if we could eliminate variations of fly prevalence by imagining a high fly prevalence evenly maintained throughout the season, the diarrhoea curve would be seen to rush up to it, and then fall away, with the typically explosive outline.

Finally, it is important to note that the diarrhoea curve—although at first falling more quickly than the fly curve—later on, however, tails out, so as not to finally collapse till about the same time as the fly curve; that is, about the time the weekly air temperature passes decidedly below 50° F. Only two of the records are however continued long enough to give any indication as to whether at this point the level of fly prevalence becomes low enough to be disregarded as a factor exerting any positive influence.

A striking fact which specially deserves to be mentioned in connection with fly-carriage of diarrhoea was noted by the writer in examining charts of the great cholera epidemics of London in 1849, 1854, and 1866. They appeared to be governed by exactly the same temperature conditions as diarrhoea and to vary in the same way with variations of temperature. Particularly noticeable was the quite simultaneous subsidence of the two diseases as 50° F. was passed. Similar temperature effects upon multiplication of the respective organisms in food or water might of course be urged in explanation. But in view of the widely differing temperature limitations of cholera in various other countries

the above facts are highly suggestive—even allowing for some confusion in diagnosis of the two diseases—of the common influence of fly-carriage in both.

Another fact, with regard to the pre-epidemic period, appears to fit in well with the fly hypothesis. It will be recalled that at air temperatures of 60° F. or a little below (cf. p. 719), the first two weeks of this period pass without any sign of increase of cases of diarrhoea; or a longer period at lower temperatures: afterwards a slight increase leads up to the main rise. Fly-counts, such as at London, 1909 and 1908, and at Manchester, 1906 and 1904, also indicate a corresponding interval or its equivalent before increase of flies occurs: and 14 days, at such temperatures, is just about the period necessary from the laying of eggs to the hatching of the first brood of flies. Thus Newstead (1907, p. 16) says: "The whole cycle from egg to perfect insect occupies, under the most favourable conditions, from 10 to 14 days; but in low temperatures the whole cycle may extend to several weeks."

In conclusion it might be stated that of the various points of non-correspondence of the fly and diarrhoea curves noted here, none of them have been found impossible of explanation, so as to be incompatible with a theory of fly-carriage; although causation is not necessarily thereby established. The same applies to the objections noted in a former paper (1908), which, with the help of the four additional sets of data since provided, particularly those of London for 1908 and 1909, mostly appear to be at least capable of explanation on the principles above laid down. An ambiguous sentence (*ibid.* pp. 36, 37) contained in the above, which was marked for revision but finally escaped notice, as to the balance of epidemiological evidence being unfavourable to the fly theory, referred, as the accompanying context suggests, to the evidence of the fly curves then available, and not to the large amount of epidemiological evidence of other kinds. The concluding sentence, which may just as aptly be applied to the conclusion of the present section, refers to the correspondences of temperature, flies and diarrhoea, as being so extraordinary "that the whole question merits the most thorough and laborious investigation" (*ibid.* p. 37).

*The suggested correspondence, both in rise and decline, of the 4-ft.
Earth Temperature tracing with the Diarrhoea Curve.*

It might be well to again insist on the fact that any correspondence of the 4-ft. earth temperature tracing with the curve of diarrhoea

prevalence, *e.g.* as regards the very frequent attainment in this country of their maxima at the same time, and any correspondence in commencement of fall (cf. Ballard, 1887—8, p. 3), are quite unessential facts as regards causation; as they wholly depend upon the mere chance agreement, in the short summers of this country, of the length of the interval occupied in rising to the acme of the temperature curve, with the length of the interval by the end of which the diarrhoea epidemic has usually ascended to its acme, and begins to show signs of exhaustion by descent of the curve (cf. 7 yrs. Lond. in Chart B, p. 720). In countries having protracted summers—judging their length by the amount of time passed above a certain temperature level, the diarrhoea may reach its maximum, and begin to decline, more than two months before the 4-ft. temperature has reached its maximum (Armstrong, 1905, p. 517).

Again, with regard to the rise of diarrhoea and its relation to the 4-ft. earth temperature, objection must also be taken to the assertion, without proper qualification, that the 4-ft. temperature furnishes a register of cumulative temperature. That may be apparent in a few seasons where an interrupted rise of temperature has taken place, and is still taking place, but where, through remissions of temperature, the 4-ft. record has once become stationary during its ascent, or has fallen, all account is lost of the amount of heat received during that check, and such irregularities occur in the majority of the short and irregular seasons of this country. The 56° F. standard certainly furnishes a sign very easily determined and very intelligible to the inexpert, in comparison with the accumulated air temperature standard, which is somewhat difficult to estimate, and to express in brief concise terms. But notwithstanding, in the former case, so many exceptions have to be explained away, and so many adjustments to be made, that it is doubtful if it possesses much advantage in these respects over the latter, even apart from its very questionable adaptation as a scientific test (cf. also p. 686, and Chart B, Lond., 1907). In records and charts of diarrhoea prevalence, where one temperature record only can be included, it should unquestionably be that of the *air* temperature. Such a preference is the one most consistent with a strictly scientific view of the matter; since the air temperature is the one most directly connected with diarrhoea prevalence, and also shows the best correspondence to the fluctuations and fall of the diarrhoea curve (due allowance having been made for the explosive contour of the latter). Both the 4-ft. and 1-ft. earth temperatures may, however, be also usefully included.

The suggested influence of the 4-ft. Earth Temperature, upon Diarrhoea Prevalence, through its suggested direct relation to Fly Prevalence.

Similar care is needed, to that enjoined above, in interpreting apparent correspondences between the fly curve and the 4-ft. temperature tracing; and any attempt to explain the apparent inter-relation of the diarrhoea and 4-ft. temperature curves, by bringing into prominence a direct effect of the 4-ft. earth temperature upon fly-breeding, without clear and sufficient warrant for doing so, tends only to re-introduce confusion into these matters.

It is difficult to understand how the latter temperature can have much influence upon the dates of *commencement* and *completion* of seasonal fly prevalence, even in the districts where there are many privies and manure-pits sunk in the ground. Firstly, the deep earth temperature is notably lower than superficial earth temperature at the beginning of summer: therefore, the first swarms of flies will not come from such situations but rather from heated manure lying more superficially; and secondly, though at the close of the season with the earth temperatures comparatively higher than the air temperatures, sunken pits would enable the development of fly larvae to continue; yet, seeing that fly pupae are generally deposited near the surface, the influence of such pits will be largely neutralized by the fact that the emergence of fresh swarms of flies will be completely subject to, and inhibited by, the comparatively low air temperature.

The primary fact of the seasonal rise and decline of fly prevalence appears to be the direct effect of the *air temperature* upon the imago or adult insect, determining its first emergence, and its final immobilization; and to a less extent upon the pupae: both of these are habitually more or less exposed to its influence. Otherwise, as regards the larvae and eggs, there is as much hot manure in winter as in summer for developing the larvae, but the lowness of the temperature in winter immobilises the adult fly, and so prevents the laying of eggs.

The almost immediate response of the numbers of flies to the fall and rise of temperature is very curious: if not wholly due to the effect of variations of temperature upon the amount of fly movement, it may possibly be due to some extent to the flies going into their hiding places, as they are supposed to do in the winter time, upon the super-vention of a cold change—especially if of a gradual kind, and emerging at once on a change to warmer weather. Otherwise, the prevalence of

any one week appears to depend upon two factors: firstly, upon the favourableness of the temperature for egg-laying two, three, or more weeks before; and secondly, upon the favourableness of the present temperature for emergence from the pupae. It so happens, therefore, that past and present temperatures may sometimes counteract each other with regard to their effect upon prevalence.

The influence of rainfall upon diarrhoea prevalence need not be discussed further than to say that its influence is probably almost wholly produced by its effect in reducing the temperature: this should, of course, react secondarily upon diarrhoea prevalence. From a few observations the writer has made, it appears that its influence in driving flies indoors depends wholly upon whether during the fall of rain the temperature indoors is warmer than without; if cooler, the flies appear to remain outside. On comparing the meteorological and prevalence charts for Mansfield, there certainly does appear to be some amount of correspondence with rainfall. It is not certain however that irregular periods of collection of the data had not a good deal to do with this (cf. also paper, 1908, pp. 12, 13).

(ii) *Influences determining the outline of the Diarrhoea Sickness Curve.*

The preceding remarks upon the mortality curve appear to apply equally as regards the effect of temperature and the exhaustion of epidemic potential, to the curve of prevalence of *all cases* of diarrhoea; the latter yielding confirmatory evidence upon these points.

A comparison of *Ballard's* (1887-8) *returns of diarrhoea sickness* for 10 years (1857-66) at Islington, with Greenwich air temperatures, bears out the conclusions on these points: epidemic explosiveness and exhaustion are particularly well illustrated. The years 1858, 1862, and 1866, show the epidemic continuing to rise for one and two four-weekly periods after the maximum temperature has been passed. In one year, 1865 the epidemic prevalence fell rapidly, while the temperature was maintained or still rose slightly, for two more of such periods. Moreover, 50° F. appeared to be the limiting temperature. The two most insignificant outbreaks, 1860 and 1862, were those in which the highest average temperature reached only 58° F. and 60° F. respectively; and for one monthly period in each. The tallest curve, 1859 (excluding the cholera year 1866), surmounted the highest four-weekly temperatures of the whole series. There were no notchings of the curves except in one instance; owing no doubt to the length of the periods taken; so

that the effects of small variations of temperature were thus not observable; although the effects of larger variations were to be seen in the ways above noted, and also in the steepness of slopes of different curves.

The *Mansfield data* will now be similarly examined.

(1) *Irregular evolution in point of time of the epidemic, in different districts and neighbourhoods.*

Although, a short time after the arrival of the air temperature at 60° F., simultaneous appearance, or increase, of cases occurred generally throughout the town, *i.e.* in 13 out of 15 localities visited (cf. p. 711); these cases were nevertheless comparatively few and scattered, and the main seasonal outbursts in the various districts did not follow, in some instances, until considerable periods had elapsed; the outbursts in different districts, again, occurring at widely different dates; and the dates of attainment of the maximum prevalence also differing to a certain extent. Similar differences are apparent between the subdivisions of a large city, when compared as regards mortality, as illustrated in the writer's former paper (1908, Chart VIII), the differences being less, the larger the divisions taken: hence the greater reliability of massive statistics (cf. also pp. 646 and 695).

Such differences may be explained in one or more of the following ways: firstly, they may depend upon the transference between different districts of specially virulent strains of infection. The main outbursts may be so determined, while the scattered low prevalence found on all sides during the season may be due to widespread strains of only ordinary virulence. Secondly, they may depend more upon such strains of infection gaining access at varying dates to centres of highly susceptible people. Thirdly, they may depend wholly upon irregular local prevalences of flies.

It may be briefly stated that, from careful observations upon this matter, a general conviction was received as to the frequent applicability of the first, and to some extent of the second; but irregular local development of fly prevalence did not appear to be an important factor; although it is quite possible that it may have frequently played such a part (cf. also p. 667).

Some details as to irregular evolution in the several districts may now be given.

The main rise in the quadrilateral did not occur till more than four weeks later than in the triangle—and this was distinctly not due to later visitation and collection of data in that area; appearing also to continue

till a correspondingly later period: a certain number of scattered cases had however occurred from as early a date as in the latter district. As regards other parts of the town, the main rises were found to occur at widely different times; but most of them, it may be noted, occurred more about the same time as that of the quadrilateral than at any other. Area III (Charts III and IV, App.) began a week later; and Area IV (Chart IV, App.) a week earlier.

Although there was good reason to believe that in several other foci there was also a large number of cases occurring as early in the season as the main rise in the triangle, yet the great contrast in date of onset, between the comparatively large and important outbreaks in the triangle and in the quadrilateral is a very remarkable fact. Differences in temperature, owing to altitude, are not marked enough to account for it: Area IV with a three weeks later epidemic being not so elevated as the triangle: and at the same time it is evident that the rule as to a fixed period of cumulative temperatures is widely diverged from in the case of the ultimate local units or foci of the epidemic wave; pointing away from a general and mechanically precise reaction of ground organisms to temperature, to case-to-case methods of spread, and also to irregular transplantation of strains of infection of varying virulence.

It is interesting to note that the groundwork of the charts between the specially virulent foci is filled up by a large number of scattered cases, which from their isolated nature, in spite of apparent susceptible material close at hand, are presumably of very low infective virulence.

The analysis of the outbreak in each of the two large districts shows great divergences in date of outburst in different neighbourhoods, and suggests even a possible perambulation of infection from one part to another. When the quadrilateral is divided into five districts to best set off these occurrences, the outburst was curiously later and later in each neighbourhood, on going north; as if spread had occurred in that direction from the few virulent outbreaks—one of which was imported as previously described—that had occurred in the first section at a comparatively early date in the season. Again, in the triangle where the distribution of the epidemic naturally occurred in three divisions (Chart V, App.), the second division appeared to derive its infection from the first, by gradual passage from the very early outbreak in α Street, along β Street, to δ Street. These may only be coincidences, but still, occurring as they do in both districts, they are worth calling attention to.

(2) *Effect of Temperature in producing variations in prevalence of Diarrhoea Sickness.*

The sickness data of a town of considerable size would of course require to be furnished in order to consistently demonstrate the influence of temperature, which as has been before suggested is of a very general kind; its effects in producing variations in prevalence, when only small districts are taken, being frequently smothered by the irregular firing off of the various component foci of infection. In the data here presented, many of the dates of attack were only approximate, the attacks being referred to indefinitely as having occurred in the middle, or at the end of, a certain week: such cases were respectively placed under Wednesdays or Thursdays, and Sundays; which gives an excessive incidence upon these days. However, many general variations corresponding with temperature can be found upon a not over-critical study of the charts. Thus, the cessation of epidemic prevalence can be made out about the time the air temperature passed decidedly below 50° F. Again, the most marked variation in temperature, during the season, was the very unseasonable rise at the end of September, following after a period of rather low and intermittent temperatures. In correspondence with this the curve of diarrhoea for both districts shows a late rise. The curve for the triangle, again, is mainly composed of three humps corresponding with three similar elevations of temperature (particularly as regards maximum temperatures) during the period of highest temperatures, from about June 22 to August 9. After the latter date there was a fall, followed by a slight rise in both temperature and cases, particularly between August 16 and 30; and then a sudden fall of cases almost to zero, accompanying a fall in temperature to somewhere about the limiting temperature of 50° F.

(3) *Evidence of Epidemic Exhaustion.*

The foregoing remarks as to the great divergence in dates of outburst in different neighbourhoods and districts may now be completed by the addition of the important observation that almost as a general rule *early local outbreaks were correspondingly early in coming to an end* or in falling to a low prevalence, in spite of the continued maintenance of favourable temperatures; and late outbreaks were correspondingly late. The conclusion suggested is that in the former case the epidemic

has worked itself out early, by exhaustion of susceptible persons, or of infective virulence in the causal organism; for there is not infrequently marked absence of cases in such neighbourhoods for the rest of the season, as well as before. In fact the entire epidemic in the two districts appears to be almost wholly made up of a number of these small local explosions, isolated in point of time, as well as of place, and each about three weeks in length.

This exhaustion in small foci is evidently then the expression in miniature, and the foundation of, the similar phenomenon exhibited in the curve of total prevalence for a large district, or for a whole city: this matter, which has already been fully demonstrated with regard to the mortality data, will also be found to lend itself readily to demonstration from the point of view of sickness data.

All the above facts are very clearly exhibited in Charts IV, V and VII, App.; the local outbreaks referred to are as a matter of fact the place and time groups discussed on p. 688 *et seq.* The two main facts to be noted are: firstly, in Chart VII, it is seen that however early the outbreak in one of these clumps was placed in the season, the clump as a whole was practically never attacked again during the rest of the year, and even solitary cases were very infrequent: secondly, notwithstanding the wholesale prevalence on every hand, 86% of the cases were not attacked a second time during the season; and 96% had no second attack more than six weeks after the first. From these two points *a conclusion as to the reality of epidemic exhaustion by exhaustion of susceptible persons is unavoidable.* It is evident that generally speaking the epidemic cannot, or at least does not, attack where it has been before in the same season. If then the infective virulence remains constant, it necessarily follows that as the season progresses the number of susceptibles diminishes, the distance between them increases, and the chance of attack consequently becomes continually smaller.

Comparing now the individual prevalences of the two large districts, it is at once seen that *the outbreak in the triangle is a remarkable illustration, on an extensive scale, of early exhaustion of the diarrhoea epidemic.* In both districts the main epidemic was limited to about the same period, *i.e.* eight weeks; but that in the triangle, commencing four weeks earlier than that in the quadrilateral, was exhausted—at least to a very low prevalence—four weeks earlier; notwithstanding the fact that both districts evidently shared the same temperature conditions. Again, it will be seen that even the quadrilateral, which had an epidemic as late as that in most parts of the town, showed weakened response to rises of

temperature as the epidemic progressed; the outbreak in the southern half being finished particularly early. And indeed the argument from small foci to large districts, and from large districts to the whole town, naturally follows without the necessity of further demonstration; particularly as it has been shown that the disease was already scattered through every part of the town from the very commencement of the season.

Moreover, all the other peculiar effects due to the interaction of temperature and the typical explosive epidemic, which were noticed in regard to the mortality curve, are also individually evident with regard to these sickness data. Thus, some local explosions, which happened to commence just as a fall to low temperatures had taken place, continued to rise notwithstanding. Cf. the explosion in the week ending August 22, on the curve of the triangle, which was wholly confined to one neighbourhood. Again, the curve, even with these limited data, is seen to be decidedly *rounded off*, in descent as well as in ascent, not exhibiting, moreover, mechanical and sharply cut reactions to temperature variations. *The tendency to a typical explosive curve, exhibiting epidemic exhaustion and all its other characteristic features, is thus unmistakably revealed.*

As regards explanations of epidemic exhaustion, other than that of exhaustion of susceptible persons, *exhaustion of infective virulence of the causative organisms* might be suggested; but in this case it might have been expected that neighbourhoods in which an outbreak had worked itself out would frequently have been re-attacked later in the season, from the spread from adjacent or distant neighbourhoods of new and fully virulent strains of infection. But second outbreaks in these clumps were exceedingly uncommon. The manifesting of exhaustion after the end of eight weeks from the rise, a period of similar length being found in different countries in spite of long-continued high temperatures in some, is however of peculiar interest with respect to the above matter (cf. p. 725). Again, the question of small local decreases in fly prevalence need not be considered, from the fact that clumps, attacked at widely different dates, were frequently closely adjacent. Moreover, as regards the four weeks earlier exhaustion in the triangle than in the quadrilateral, it is difficult to believe that the fly swarms were exhausted or were willing to leave so attractive a district as the triangle thus early in the season; or that if they were, that swarms from neighbouring districts would not have freely migrated into it to fill their places. *Exhaustion of epidemic potential apart from decrease in flies, which was*

demonstrated without doubt with regard to the relations of the mortality and fly curves (cf. p. 727 *et seq.*) is thus demonstrated with almost equal certainty from the sickness data.

Finally, it might be noted that the definite amount of acquired immunity found in Sect. III, 5, to follow attack, along with the above facts, suggests that exhaustion of susceptible persons was by far the most important factor in the question. Some explanation is, however, required of the bald statement given above, that the early collapse in the triangle or in the southern part of the quadrilateral was due to exhaustion of susceptible persons. It is not for a moment suggested that there were not numbers of persons left unattacked who would have succumbed to the disease had fair opportunity been given. That is evident from its behaviour in π Street, where, after a moderate prevalence similar to that in other parts of the district, the disease, so to speak, broke out again, 25 out of 58 persons in houses 4—16, *i.e.* nearly half, being attacked. A similarly severe incidence was also found there in 1909. A high incidence, similar to that found in 1908, was also found in γ Street in 1909. It appears then that, in certain dirty parts of the town, almost everyone might be attacked once in two or three years. On the other hand, there is reason to believe that in clean parts many people may go on from year to year without having the disease at all: and yet they must necessarily possess less acquired immunity than the people who are so frequently subject to attack. It is evident then that, as suggested before, *the question of exhaustion of susceptibility of a population is altogether a relative matter.* It does not depend wholly upon individual immunity, and upon the virulence of the infection, but upon the *total resistance* offered by these and *various other factors*. Thus, in a clean area, the resistance to spread is so great, that whatever infection enters, dies, so to speak, an early death; being able to reach only those who present an extraordinarily high degree of susceptibility; and probably even missing the greater part of these, from want of ready means of dissemination. In a dirty district, however, free course is given to the infection. Nevertheless, as evidenced also in the dirtiest parts of the triangle, there is a limit here also, and the outbreak will generally fail and disappear for the rest of the season before half of the people are attacked.

Epidemic exhaustion is particularly well shown in the early and heavily attacked α and β Streets (Nos. 1—35 and Nos. 41—63). Only one case occurred, in the season, after August 19, while in the quadri-

lateral nearly half the attacks, and in the whole town nearly all the diarrhoea deaths, occurred after this date. In *a* Street, where the houses were very dirty, and where the earliest explosive outburst occurred, 14 persons had been attacked in the ten houses, and the epidemic was practically over for the rest of the season by July 15, before the hottest of the summer, which preceded the outburst in the quadrilateral and in District III, had arrived. Further, the question of immunity might be expected to have some bearing upon determining the distribution of attacked houses into clumps, and also to qualify the results of many of the analytical tables throughout this paper. There has not been time to completely follow out all these matters; but from a cursory examination, it appears that no material qualification of any of the conclusions arrived at would be caused thereby.

(c) *Conclusions as to factors governing Epidemic Prevalence.*

(1) *The "Ground" theory versus the "Fly Carrier" theory.*

As regards the various interpretations, above given, of the phenomena both of epidemic rise and decline, an attempt will now be made to decide *which combination of such interpretations most closely coincides with the facts at present available.* The matter is rendered somewhat complex by the introduction of the opposing theories of ground infection and personal infection; and the various possible interpretations have, for the sake of clearness, been tabulated below.

A.—The temperature phenomena of the two periods may be interpreted in one or more of several different ways; as follow:—

The pre-epidemic period:

(i) As being occupied in maturing a high degree of infectivity in a ground organism.

(ii) As necessary to the production of effective swarms of fly carriers.

The epidemic period: the temperature notchings and limitations may be interpreted—

(iii) As a direct effect upon the infective virulence of the crop of ground organisms from which all epidemic cases might prove to be directly derived.

(iv) As a direct effect upon the infective virulence of the organism, during the brief interval of exposure, in passage from person to person.

(v) As a direct effect upon the prevalence of fly carriers.

B.—Epidemic Exhaustion may be interpreted in one or more of the following ways:—

(i) As exhaustion of infective virulence of a ground organism, as it lies in or on the ground: infectivity being a function with seasonal limitations, like as in the flowering of plants.

(ii) As exhaustion of infective virulence in an organism, personally transmitted.

(iii) As exhaustion of susceptible persons; whether the organism be derived from a ground, or from a personal, source.

Before proceeding to discuss the relative values of the above hypotheses a brief recapitulation will be made of the few facts of the disease, the certainty of which the evidence of this or of other enquiries might claim to have unquestionably established.

A Statement of Known Facts.

(1) There is a constantly controlling and facultatively inhibitive force, exercised by temperature (cf. pp. 719, 739, etc.).

(2) There is exhaustion of epidemic potential, apart from the decline of fly prevalence (cf. p. 727 *et seq.*).

(3) There is undoubtedly a good deal of acquired immunity in the population; and this will account for some part of the phenomenon of epidemic decline (cf. p. 739 *et seq.*).

(4) There is undoubtedly a good deal of transmission of infection from person to person (see p. 715, Summary).

(5) There is widespread infection to be found from the very beginning of the season, that is, at least from the end of the pre-epidemic period (cf. p. 711).

(6) There is also a definite pre-epidemic period of favourable temperatures, during which diarrhoea appears to undergo practically no increase—at least at all comparable to the rate found at the time of the main rise (cf. p. 719).

To these facts of positive import may be added a few of a negative character.

(7) We have no knowledge, at present, of such occurrences as that of a direct effect of temperature upon infectivity during transmission from case to case (see *A* (iv) above).

(8) There are, at present, few facts to support a theory as to exhaustion of infectivity, during repeated passage of organisms from person to person throughout the season (see *B* (ii) above).

(9) No evidence was found in this paper adverse to the fly carrier theory. On the other hand a great deal of indirect evidence was obtained in its favour. The extent of correlation of the prevalence curves of flies and diarrhoea appeared to be quite compatible with the fly theory (cf. p. 727 *et seq.*).

In view of the first two negative considerations just mentioned, two of the possible explanations on p. 743 *et seq.*, viz.:—*A* (iv) and *B* (ii), may, at present, be practically excluded: and *with the exclusion of theory A (iv) the possibility of direct personal infection explaining the whole phenomenon of prevalence is also excluded.*

The question is thereby rendered much less complex, and *the choice is practically left between two principal combinations*, which are as follow:—

(1) A combination of explanations *A* (i), *A* (iii) and *B* (i); which is, in effect, the “ground” theory.

(2) A combination of the remaining explanations *A* (ii), *A* (v), and *B* (iii), which is, in effect, the “fly carrier” theory. Several other combinations of the theories tabulated on p. 743 can also be made; but they are not so likely to furnish the correct solution.

From what has been said in preceding sections of this paper, it is evident that there are not perhaps sufficient facts available to decide finally between the above two principal theories; the arguments for and against which are fully reviewed on p. 715 *et seq.* A previous remark might again be repeated here:—*everything waits upon a demonstration of the precise relations of fly prevalence with diarrhoea.* A theory such as the ground theory must necessarily rest for proof upon the exclusion of other more likely theories; and a demonstration that fly carriers are responsible for a really considerable part of the epidemic cases would of course tend to speedily dissolve the claims, and the necessity for the existence, of the former hypothesis.

On the other hand, it is possible that both influences take some part in the matter; and from *the piled-up mass of argument in favour of case-to-case infection*, on p. 715 *et seq.*, it is impossible to avoid the conclusion that, already, the ground theory must be profoundly modified to accommodate these facts. But here a grave difficulty arises; for, the greater the amount of case-to-case infection admitted the greater difficulty is there in explaining the profound variations or limitations of prevalence produced by temperature; for a direct effect of temperature upon the infectivity of the organism during transmission (see *A* (iv) above) does not at present come within the bounds of known and proved

facts; and we are thus driven on, willingly or unwillingly, upon the fly carrier theory, which has already been shown capable of satisfactorily explaining the whole matter, and which is the only one appearing, at present, at all likely to do so. On the other hand, though the theory of case-to-case infection should be completely demonstrated, the precise method of transmission is a question altogether apart. Moreover, the evidence for fly carriage is only of an inferential kind, and *we dare not therefore accept the fly theory without certain confirmatory evidence of a direct kind*, such, for example, as that to be obtained by the three crucial tests outlined on p. 708. Again, the possibility also still remains that ground influences may play a definite, if only a minor part, in producing a seasonal increase of infectivity in the organisms which the fly carrier has the task of distributing.

(2) *The "Bacterial Content of Food" Theory.*

The bacterial content of food has been shown to vary directly with the degree of temperature (see p. 680). The fact, however, that the numbers of diarrhoea cases do not vary evenly at all times of the season with the temperature, does not do away with the possibility of the diarrhoea prevalence being largely determined by the bacterial content of food, where the causal organism is supposed to be a specific variety, not of general distribution. Thus, at the beginning of the season, a considerable *pre-epidemic period* might elapse during the multiplication and spread of the specific organism, before appreciable dissemination of infection was produced. Again, later in the season, the phenomenon of *epidemic exhaustion* might represent, in addition perhaps to some exhaustion of susceptible persons, exhaustion also of the function of infectivity, or of multiplication, of the specific organisms. As regards the evidence for case-to-case infection, it will be recalled that the major part of that evidence rested upon the grouping of cases in point of time and of place; and this is quite consistent both with a "bacterial content" theory and to some extent with a "ground" theory, where the causal agency is a specific organism not distributed in a widespread manner, but limited to certain small foci. Bacteria might gain access to the food both by *dust*, *flies*, and human agency—the theory admitting of a large amount of case-to-case infection. Variations of prevalence with temperature could be explained by acceleration or retardation of multiplication, of the specific virus in food; as well as partly by effects upon fly prevalence. The moderate probabilities

existing as to the correctness of this theory; and, to a less extent, of the ground theory; indicate the need, at present, of some reserve in drawing final conclusions as to the causation of diarrhoea.

(3) *Some other Theories.*

A few agencies might here be briefly referred to, such as *heat* and *fruit*, which may possibly exert influences, at least of a general kind; and which were dismissed in an earlier part of the paper (cf. VI, 3, p. 683; and p. 715) as factors not exerting any special influence in the causation of diarrhoea. If heat has no direct disturbing influence upon the digestive organs in summer, it at least greatly stimulates fermentation or kindred processes in food, particularly in fruit and milk: and the digestive disturbances due to the latter may have not improbably a good deal of influence upon diarrhoea prevalence, at least upon the total amount. It is also quite possible that such digestive disturbances may be the means of lighting up much old infection in chronic and recurrent cases at the beginning of the season.

The phenomenon of *symbiosis*, again, always contains interesting possibilities; and, although the specific organism of diarrhoea may not itself be derived from the ground, yet a symbiotic revivification of its infective virulence may be produced, within the body, by the entrance within the latter, at the beginning of the season, of certain saprophytic ground organisms, which have themselves been exposed outside the body to, and modified by, the maturing temperature influences of the pre-epidemic period.

In conclusion, however, it is a question whether it will not be finally demonstrated that the influences determining the epidemic prevalence of diarrhoea are referable, not wholly to one, but to a number of, interacting factors. Thus, whether there is, or is not, maturation of a ground organism, there is not improbably, in any case, a certain amount of fly carriage, of multiplication in food, and of direct personal infection; as well as, possibly, some exhaustion of infectivity of the causal organism, beside the known and proved exhaustion of susceptible persons. It is important, again, to note that of the various theories as to method of spread, the "*fly carrier*" theory is, at present, the one best able to stand alone as a complete and all-sufficient explanation of the facts at present available.

It may further be noted however that, as regards the importance of the part played by cumulative temperature effects or fly prevalence, in

starting the epidemic, there is always the possibility that the influence of these factors may appear to be more important than it really is. The part they play may be one of minor importance; simply providing the match that lights up the conflagration in a great mass of susceptible material. The explosive violence of the diarrhoea epidemic, perhaps second only to that of measles amongst the commoner infectious diseases, should be here recalled, and particularly the violent up-rush in hot countries; and the immediate falling away, as if the epidemic had over-reached itself, occurring months before midsummer is reached (cf. Chart B, p. 720, Melb.). Due importance must be therefore given to the great quantity of susceptible material that has been accumulating since the preceding epidemic, or to the possible alternative theory as to gradual recovery of infective virulence of the causal organism. It is not certain, however, that the rise in late seasons occurs in greater force on account of being greatly delayed. Conversely, the possibility might also be considered, whether, after a succession of seasons in which no epidemic has occurred owing to the complete absence of the necessary temperatures or again of fly prevalence, the diarrhoea prevalence might not force its way up in spite of the almost complete unfavourableness of the latter factors, owing to unwonted great accumulation of susceptible material or to the complete recovery of a high degree of virulence. It might thus be advisable to provisionally consider the influence of temperature or fly prevalence as to some extent a relative rather than as altogether an absolute deciding factor of the epidemic prevalence of diarrhoea.

VIII. PREVENTION AND TREATMENT.

Were it possible to sum up the essential features of diarrhoea in a single sentence, without risk of misunderstanding, it might be said that epidemic diarrhoea is a disease of *young, healthy and dirty* families. The apparent paradox which the second of these epithets presents has already been explained on p. 628: in the same sense as that in which the term is applied to certain acute specific diseases which notably affect a large number of healthy young persons, diarrhoea also is a "disease of health." The mortality alone is, however, largely confined to weak and ailing infants. From this latter fact the argument has been deduced that preventive measures tend only to retain within the population a large number of the "unfit," whose loss would rather be a gain than otherwise to the community. But it is only necessary to point,

in answer to this, to the still large minority of cases which succumb to severe infective diarrhoea, who are physically of the soundest (cf. p. 629); and to the after effects in those who recover. That *diarrhoea calls urgently for measures to limit its yearly ravages* is evident from the three following important economical considerations:

(1) *It is the cause of a huge infantile mortality*; and moreover there is reason to think that the many deaths certified as due to "convulsions," "dentition," "marasmus" and other like ailments which, it is significant to recall, also show marked seasonal variations parallel with those of diarrhoea, are very often actual instances of, or at least closely related to, diarrhoea.

(2) *The after-effects it leaves upon those infants who recover is probably an equally serious consideration.* Firstly, it should be noted that in the two areas an average of half the babies in each of the first three years of life were attacked during 1908, a year of only moderately high prevalence; so that it might be fairly assumed that almost all the babies born in these districts had diarrhoea at least once before reaching three years, and many of them on several occasions: a wholesale incidence which recalls the similar wholesale affection of the children of the coloured races with malaria. Secondly, serious impairment, such as fibrosis, of the organs of the body is said to follow and to make itself apparent in after life; which might indeed be expected from the serious lesions described by Ballard (1887-8, p. 15) in the cases examined. Thirdly, all this is well borne out by Newsholme (1909-10), who found that towns having high infantile mortality also had high mortalities at the later periods of life.

(3) *The great economic loss due to the illness and its after-effects in adults*, who are frequently compelled to remain away from work for long periods (cf. p. 622); but not the least important, from the writer's observations, are *the immediate after-effects—the low state of vitality induced at the close of the autumn*, leaving both adults and young children an easy prey to the bronchitis and pneumonia of the cold weather which immediately follows.

Finally, to the above observations might be added that of the wholesale incidence of this loathsome disease in certain parts of the country, *e.g.* the yearly occurrence of about 3000 cases in a population of 33,000. Better warrant than such facts offer could hardly be desired for the institution of wholesale preventive measures in a disease which is at once the scourge and the great sanitary reproach of so-called civilized lands.

1. *Treatment of Diarrhoea.*

As regards treatment, the various adjustments of the diet are useful, in great measure perhaps because they serve to pick out cases of irritative digestive disturbance, where the specific element is either little in evidence or completely wanting: and such procedures as infusion of fluids where there has been great watery discharge, and washing out of the bowel, are doubtless also of service. But where a severe typical attack has to be dealt with it is probable that, as in the case of specific diseases generally, it has to run its own course, and the hope of the treatment of the future must lie in some anti-specific procedure, such as preventive inoculation or administration of curative serums. Above all things the discovery of a powerful antitoxin would be gladly welcomed. The cloudy swelling and marked degenerative changes in the kidneys and other organs (Ballard, 1887-8, p. 13), along with the great depression, point to the production of a powerful toxin, to whose agency the dangerous accompaniments of the disease are largely attributable. The sharpest and most serious attacks last only for comparatively few days, and several injections of an efficient antitoxin would serve to carry the patient triumphantly through. But not only would such an antitoxin be invaluable for saving life, it would also have an enormous economic value in alleviating the suffering and prostration of non-fatal cases. It was surprising to meet so large a number of adults, especially men, who had to stay at home from work on account of diarrhoea, the length of the detention being often a week or a fortnight. The prostration was also great, even to the point of danger to life; and in such cases one could not help thinking an efficient remedy, such as an antitoxin might provide, would have been a great boon.

Whatever advances in treatment may be reserved for the future, there is some reason to hope that a great deal can in the present be accomplished in the way of prevention.

2. *Prevention of Diarrhoea.*

No attempt will be made to deal exhaustively with this question, but merely to throw out a few suggestions which have come most into prominence during the course of the enquiries.

(a) Notification of Diarrhoea Sickness.

Apart from considerations as to the value of the opportunity given by notification for remedial measures, there is the practical question as to whether any really large part of the cases would thus be brought to light. This was another interesting matter kept in view throughout the enquiries of 1908. I found that medical advice was sought in 49 out of a total of 390 separate attacks in the two districts, or in 12%. The data on this point were not quite complete, but the proportion could not have been very much more: and that in districts where most of the people are in medical clubs, and are in the habit of seeking medical advice pretty freely! This is in itself confirmatory evidence of the indifferent light in which the gravity of the affection is viewed amongst the classes who suffer most from diarrhoea. Only this one-eighth of the cases applying for medical relief would thus, in the usual course of things, have been notified. But although only 8% of attacked persons over two years of age were found to apply to a doctor, yet in this, and perhaps most other towns, probably many times that number apply to a chemist, diarrhoea mixtures being in great request during the hot summer. Thus it is evident that any attempt to obtain full notifications of all cases might need to include notifications by chemists. On the other hand, the financial burden imposed upon local administrative bodies by notification of all cases, in towns where the latter might include 10% of the whole population, is practically prohibitive of the procedure; and, again, until this wholesale incidence is reduced to more moderate proportions it is doubtful if the money would not be much better spent upon measures directed against household dirtiness and, perhaps, fly nuisance. *Notification of infants only* might, notwithstanding, be completely practicable and eminently beneficial, as *under two years of age* they only include $\frac{1}{5}$ th of the total number of cases, and since it is amongst infants that the greatest susceptibility, as well as most of the mortality, is found. It is true that, with a view to eradication measures, notification of older children and adults is desirable; for the passing on of infection from the parents to the young children must be borne in mind. Yet, on the other hand, recalling the great attraction for diarrhoea of houses containing infants, if the special incidence upon infants could be satisfactorily dealt with, the strongest predisposing cause for spread of the disease would be removed.

As to the age limit of notification, it is obvious that, in the first place, cases under 12 months, amongst whom the high mortality occurs,

should certainly be included. Children of from one to two years, however, also present special reasons why they should be included, since their susceptibility to attack is greatest of all, and they are apparently the chief means of carrying on infection from one season to another: a fact of the greatest importance, considering their very frequent association with infants under 12 months. The fact that between two and three years also the susceptibility is very high, and still as high as in the first year, is a good reason for including children up to three years. *Where, however, the chief end in view is the lowering of infant mortality, or where it is necessary to proceed on economical lines, notification of those over one or two years could quite satisfactorily be made conditional upon there being an infant under one year present in the same household: the protection from infection of those under 12 months being the main object to be kept in mind.* As a practical point it is interesting to note that of all separate attacks in the two areas in those under two years of age, out of 74 instances, medical advice was sought in 27 %, as against only in 8 % of those above two years of age.

The Notification of Births Act and the appointment of Health Visitors has furnished various ways and means of following the health of the infant from birth onward; but, notwithstanding, since the Health Visitors' calls are necessarily separated by intervals of at least some weeks or months, it is evident that some means of securing more prompt notification to the Health Authorities of the occurrence of diarrhoea amongst young children should be arranged.

Finally, although partial notification may be unsatisfactory as regards comprehensive preventive measures; yet the lessons imparted in the feeding, caring for, and protection of infants from infection, cannot fail to have a beneficial educative effect upon the mother, if not at the time for the saving of the infant attacked, at least as regards preventive measures in the future.

(b) *Isolation.*

(1) *The precautions adopted with regard to the isolation of attacked persons* will depend to some extent upon the final verdict as to the relative importance of the parts played by direct personal infection and fly infection. It would be altogether premature to attempt to formulate final methods of procedure until then. Nevertheless, in the meantime, a number of precautions might be recommended provisionally, particularly as in any case they must follow along very similar lines. In the first

place, it would be wise, in any case, to separate the sick and the healthy as far as possible from one another. Due allowance must be made for the fact that the writer's conclusions upon this subject are not yet complete and mature, and may yet be subjected to very radical modifications: but in accordance with present conceptions of the disease, if asked to guarantee the preservation of an infant charge from infection I should not for a moment think of allowing it to remain in the same house as a person attacked with epidemic diarrhoea, the risk of infective matter gaining access to it, whether directly or indirectly, being too great, even with the adoption of all ordinary precautions. Thus, when diarrhoea occurs in a household a susceptible infant should be sent away with its nurse. In the class of households above dealt with, where this would not be practicable, a large degree of isolation is still possible. Thus, the affected person and unaffected infant might be restricted to separate rooms, at the very least when motions are being passed. The Health Visitor might also advise special precautions with any young children affected in the early spring, who appear to be chronic or recurrent cases likely to provide centres of infection at the beginning of the diarrhoeal season.

(2) *Backyards-in-common* should also be discouraged in all house-planning schemes, even on general sanitary considerations alone. Such yards afford too great facilities for intercourse between the members of neighbouring households, particularly between those of tender years. Not only diarrhoea, but other infantile disorders of non-school age, such as whooping cough and measles, undoubtedly owe their possibilities of spread largely to this means, and the writer has made some attempt to investigate this matter. As regards even diphtheria and scarlet fever, I have noticed that in such yards, in summer, the amount of close association of children is almost as great as in the course of their attendance at schools. The ideal of preventive work against infectious diseases of all kinds is to increase the distance between the various units of the population as much as possible, and this is best attained by securing in the first place the greatest degree of isolation of individual households from each other. In addition, there is the moral effect, with the greater privacy and independence enjoyed: all the households in one yard are apt to sink to the same level of carelessness.

(3) *The choice of a residence* is another matter of great practical importance; particularly as moving from house to house presents no difficulty to, and is almost an annual occurrence with, many people of this class. In cases therefore where the parents express some special

anxiety upon the matter, the practitioner should certainly advise a choice of residence where the mass influence, firstly of babies (cf. p. 651), and secondly of dirtiness (cf. p. 654), can be avoided. The eastern half of σ Street, with a complete absence of infants, and a high standard of cleanliness, illustrates the complete practicability of choosing such sites.

(c) *Cleanliness in the household.*

It has already been remarked how great a scourge a dirty household may become to the neighbours in the matter of diarrhoea infection. The comparison of "clean" and "dirty sections" (cf. Table XX *b* and p. 654) showed that, amongst the cleanest of houses not containing infants (indices 1 and 2), twice the proportion were attacked in "dirty" as in "clean sections." In other words, *seven of these houses, themselves models of cleanliness*, out of a total of 33 affected households, *would not have been attacked at all, if their neighbours had been reasonably cleanly in their habits*; that is, after something has been allowed for the greater proportion of houses containing infants adjacent. The writer has felt it to be little less than a tragedy, in the following up of infantile mortality, to discover deaths from diarrhoea amongst poor but worthy people, where the cleanliness of the household and care in feeding are beyond all praise, but where the filth and accompanying disease of dirty neighbours has again and again rendered it impossible to rear a healthy offspring. Society has a right to protect itself, and in the near future will no doubt do so, against this danger from dirty people, even to the extent of compulsory legislation. The recent legislation leading to the organised visitation of homes by specially trained nurses, in connection with school medical work and also with reference to the care of infants, shows the willingness of the public to have their attention turned to such matters. It is interesting to note the present large administrative outlay upon cleanliness of children's heads, and to compare the relative benefits obtained from the latter with the great economical advantages which must result from radical treatment of household dirtiness—the rank soil, so to speak, in which alone diarrhoea can develop into the present wholesale scourge.

Allusion has already been made to the comparisons (pp. 663–4) which public health writers are frequently in the habit of drawing between different towns as regards their diarrhoea mortality rates and the accompanying provision of w.c.'s or pail closets, and recently the

central authorities have published matter of that kind with a view to drawing attention to the laxness of certain local authorities. Such comparisons usually include the factors of sanitary accommodation, and other generally recognised ones upon which official data are commonly available. Density of the infant population, and differences in case-mortality, have been noted above (p. 663) as two others to which attention should be called; also perhaps fly nuisance; and lastly, there is the important factor of household dirtiness. The addition of the latter perhaps makes the list of factors bearing on diarrhoea mortality almost complete, and it is interesting to speculate to what extent the establishment of local coefficients, or standards, for each of these factors could be systematically made, and thus a useful gauge of the local relations of infantile mortality and sanitary conditions be always at hand. A sanitary survey of towns throughout the country as regards household dirtiness, carried out if possible by the same observer, seems quite practicable, judging by the success and rapidity with which estimates were arrived at at Mansfield, and which were afterwards satisfactorily borne out by averages founded on the data of individual houses: and if the factor be as important as this enquiry appears to show, the establishment of a local coefficient, even if only made once for all, should serve many useful theoretical and practical purposes.

Inspectors are necessary whose duties should be largely those of supervision, directed to *the proper working* of the various sanitary fitments of the household. Perhaps a lacuna has hitherto been admitted here in the carrying out of inspectorial work. Having applied himself to the remedying of all kinds of structural defects and the institution of suitable drains and sanitary conveniences, the sanitary inspector has perhaps been inclined to leave these things thereafter to work themselves; but that they certainly will not do, in the slum portions of a town! It is not too much to say of localities with which the writer is acquainted, that the institution of water-closets in the dirtiest parts of the town is not a success! Full reasons for this have already been given in Sect. V, 3. Inspectorial supervision is therefore required; limited, if so desired, to the diarrhoea and typhoid seasons; and directed to securing proper working and due cleanliness of sanitary conveniences, particularly the removal of that film of faecal material which probably serves more than anything else to nullify the advantage of the water-closet over the pan closet.

A little gentle coercion might also be applied in regard to two other important matters: checking the careless disposal of the faeces

of young children, above infant years: and enjoining the regular removal of food from the table between meals into a suitable pantry.

Inspectorial observations with regard to such matters can usually be made in these small houses without crossing the threshold of the dwelling; and, as a matter of practical detail, it will speedily become apparent that there are not a large number of houses which it will be necessary to trouble with systematic visits. Unnecessary intrusion of inspectors into the home is of course undesirable, but in this matter it is a question of the undoubted right of the public to protect themselves against the breeding of a deadly disease at their very doors.

It is not out of place here to refer briefly to the chance of securing general improvement in matters of household cleanliness, and the possibility of successfully inculcating such knowledge to the school child. A striking fact, which the writer regards as of the highest practical importance, is that dirty or clean housewifery almost invariably runs in families. That is to say, the clean housewife as a rule will be found to have had a clean mother; and her daughters, in turn, will almost certainly maintain similar cleanly habits. So very few exceptions were found to this rule, in numerous incidental enquiries, that it appeared probable that early training was powerful enough, more often than not, to overcome irregular or atavistic tendencies to laxness in this respect. There is every reason therefore to expect the very best in present and future generations from the persuading of the school child into cleanly ways, and from the inculcation of the appropriate feelings of shame, or of emulation, in connection with such matters.

A convenient opportunity for instruction of the schoolgirl in matters of household cleanliness might be found in the projected lessons in mothering. The suggestion has been made that the public crèches offer very great facilities in this connection. Their establishment and extension into a kind of infant bureau is primarily an absolute necessity in the conservation of the infant life of large towns. But their usefulness may be greatly increased by adapting them as centres for the instruction of schoolgirls in mothering. It is unnecessary to enlarge upon the great interest young girls would naturally take in lessons of this kind; particularly if actually entrusted with the care of a baby, with its feeding, and with the cleanliness of its cot, clothes, and the part of the crèche in which it is located. No better opportunity for lessons in household cleanliness could be desired; and after all, the want of cleanliness in connection with babies, it seems, may prove to lie at the root of the question of diarrhoea prevalence.

Where, at the threshold of the home, the work of the supervising inspector might be advisedly relinquished, the work of the health nurse should be begun; and the chief of her duties for some time must be the education of the public, particularly of the young mothers.

(d) *Education of the Public.*

The popular apathy and ignorance with regard to this disease has already been discussed at length (cf. Sect. III, 1, p. 621). The chief points upon which instruction is required are the following:

(1) Instruction of the public as to the *specific* and *wide-spread* nature of the disease: its great economical disadvantages: the erroneous nature of popular theories as to fruit, teething, etc.; and the evil effects of the indifference resulting from connection of the disease with such trivial causes.

(2) The disease is *infectious*: a fact at present quite unappreciated.

(3) Infection is conveyed by the *stools*: risk of faecal pollution cannot be therefore too carefully shunned.

(4) The disposal of *faecal material in children* not old enough to use the w.c. requires the strictest attention—*especially in the diarrhoea season*.

(a) The child's chair should be placed in the w.c., or in some unused room apart from the general household, and the child trained to use it there.

(b) Amongst younger children, where the use of the napkin has been discontinued, the latter should be replaced during diarrhoea attacks, so as to reduce the possibility of accidental pollution of floors to a minimum.

(c) The napkins should be put in to soak in the w.c., or at least not in the scullery or living room, where contact may be established between them and food utensils.

(d) The napkins should always be completely covered with water, and the vessel itself covered over—disinfectants might here be habitually used.

Similar precautions should be adopted in soaking the soiled bedclothes, and in the cleaning up of polluted floors.

(5) Breast-feeding, and the proper care of food, are most important.

(6) Proper care of the external sanitary fitments of the household is essential.

N.B.—The above instructions are of course somewhat in advance of, and are not to be taken as, the approved conclusions of this paper. They, however, have doubtless as much warrant for their provisional adoption as *e.g.* the widely spread injunctions as to boiling of milk. And there is ample proof of the readiness with which, by a few years of persistent teaching, the public can be brought round to accept new standpoints of this kind, in the fact of the almost general adoption, in this town, of the latter procedure, and of the modern feeding bottle. It is important to note how much the co-operation of the general practitioner has meant in such matters.

Some explanation is here necessary of the fact that it has been assumed throughout this paper that communicability is exclusively concerned with infection from the dejecta of attacked persons, without any direct proof having been given. The difficulty of imagining any other mode of infection in the circumstances, and the indirect support of all the facts of personal infection here detailed, appear to justify this assumption, at least in the present state of our knowledge. During the enquiry no effort was spared to trace out other possible modes of infection and unpromising clues such as concurrent sore throat were carefully followed up, bacteriological examinations being made in two of such cases (cf. p. 623).

(e) *Breast-feeding, and the Care of Food.*

Everything that tends to the cleanliness and sterility of food, including the boiling of food and protection from dust and flies, must present a bar to the ingress of infection. The boiling of milk is a question apart, owing to the implication of matters not clearly understood relating to destruction of vital properties and alterations in digestibility. Perfectly fresh milk is probably better left unboiled: there seem to be protective virtues in cow's or goat's milk taken directly after milking akin to those in human milk taken at the breast. The keeping of these animals therefore for the feeding of infants is, next to the engaging of a wet nurse, the most reliable preventive measure that can be taken; the former being generally the less expensive procedure. "Stale milk," on the other hand, that is, milk which has lost its first freshness, is perhaps better boiled, but it would be much better not to give it at all. Attention must again be called to the protective influence of milk taken at the breast—a fact doubly accentuated. Thus, up till 9 months of age, it appeared that a breast-fed child could ingest almost

unlimited quantities of household dirt with more or less impunity. Again, there is the curious fact in Table XIX *b* that breast-feeding saved more infants from attack in the dirtiest than in the cleanest houses. If then the necessity of having the milk newly-drawn is as great as it appears to be, measures directed to securing the immediate delivery of newly-drawn and unaltered milk would take precedence over all other processes aiming at purification or preservation; and the writer is tempted to suggest that of all the various systems of milk-supply with which he is acquainted, none more closely approaches this ideal than that which he has seen in one of the old suburbs of Paris—the goatherd leading his flock and milking them at the customers' doors. From the point of view of the infant, at least, nothing better could be desired, except that the first and last milks differ greatly in the percentage of cream. There is only one alternative to this—strict supervision and prompt delivery of milk, several times during the day, to houses containing infants under 12 months of age. To accomplish this successfully, public control of the milk-supply would probably be necessary. The wholesale prevalence in the quadrilateral district, notwithstanding the complete provision of water-closets and the habitual boiling of milk, is a sufficient commentary upon the fact that the institution of these procedures still leaves the principal causative factors of the disease practically untouched.

(*f*) *General Sanitary Measures.*

All the numerous sanitary measures dealing with the general planning of houses and their sanitary arrangements must certainly tend to lessen the incidence of diarrhoea. Thus, water-closets should undoubtedly give better results than pan closets or privies were they used in a careful and cleanly manner. There is a popular saying to the effect that dirty people can turn the best appointed house into a pig-sty; and it is evident from the results of this enquiry that this kind of social lapse is not without grave epidemiological significance.

The rapidity with which people in these mining and manufacturing towns of the midlands—where, incidentally, diarrhoea reaches its greatest prevalence—can turn fine blocks of newly built houses into what is virtually slum property, is little short of amazing. The conversion of the unused front sitting-room into a swimming pool for domestic birds has been noticed; as well as the refusal by a family, through laxity or ignorance, of the use of the water-closet, the pavements around the house

being preferred. It has been thought necessary in several parts of the paper to detail a few of these persisting barbarisms, in order to avoid misunderstanding by those whose experience may be wholly confined to the cleaner rural counties of the south.

At the same time the writer would like to make it clear that Mansfield itself comes far from heading the list of the neighbouring midland towns as regards infantile mortality or household dirtiness. On the other hand, the midland mining towns are very much alike in these matters, a fact depending on the very similar characteristics of the collier population throughout. Of these towns it might be said that it is the people that live in the houses, and not the houses themselves, that are at fault, the latter being generally fairly new brick dwellings and built according to recent bye-laws: being constructed again on a very similar plan throughout the mining districts.

The essential importance of the question of dirtiness is evident in the fact that it was found to apply in the whole field of sanitary provisions both within and without the house; as regard the working of the w.c. (cf. p. 660); the yard-paving (cf. p. 657) and drains; in the care of food (cf. p. 656); and as regards faecal pollution within the house (cf. p. 656 *et seq.*).

All sanitary measures bearing upon the prevention of diarrhoea may therefore be arranged antithetically under the two following headings:—

(1) *General measures under public supervision*: with regard to proper house-planning, including suitable places for the storage and protection of food; satisfactory drains, w.c's, and ash receptacles; pure water, milk and food supplies.

(2) *Particular measures within the home, at present left entirely to the caprice of the householders themselves*; relating to the satisfactory working and application of the above general provisions, to the habits of living, and to the management of persons attacked.

While it would not be suggested that one iota should be abated of the many and various general sanitary measures that have over a course of several decades been put forward and applied in this disease, there is almost sufficient justification for declaring that *their beneficial influence may be completely neutralized*, and diarrhoea may rage with undiminished violence where there are

(a) Dirty and careless habits of living; including carelessness with food.

(b) Want of care in isolation of attacked persons and in the handling and exposure of their stools.

Again, the above facts might be profitably considered from another standpoint, the antithesis in this case being made between *public cleanliness* on the one hand, and *private cleanliness* on the other. Thus, by such general measures as the above the householder has been provided with clean air, clean water, milk and food supplies, and with arrangements for the cleanly removal of all waste matters. But it is not sufficient to merely place these benefits at his door: it is necessary not to forget the important part assignable to private cleanliness: for dirty householders, if left to themselves, are able to rapidly and effectually undo all this public labour, grossly contaminating all pure supplies received into their homes.

To introduce the topic of "private cleanliness"—including cleanliness of the household and also of the person—is to open up a department of hygiene of immense extent. That it is also a still largely undeveloped field is suggested by the fact that one disease at least—epidemic diarrhoea—has been able, notwithstanding the adoption of numerous sanitary reforms, to securely maintain its footing, and also by the fact of the large number of previously unsuspected evils only now being revealed to the public by the institution of regular home visitation in connection with the prevention of infantile mortality and with the medical inspection of schools. It is important to note the long list of evils attendant upon dirty and careless habits of living. Thus, within the home there is the increased spread and higher mortality of most infectious diseases, particularly whooping cough, measles, diarrhoea, and other infantile complaints; the septic complications of scarlet fever, diphtheria, and of advanced phthisis are also referred to similar causes. Again, with regard to personal cleanliness, one fails even yet to fully comprehend or mentally fathom the depths of uncleanness and neglect evidenced by the verminous state of the majority of the children in the slums of the large cities; and we may go even further and include matters of still more intimate personal concern, such as the question of oral sepsis from neglected teeth, with the vicious circle of throat troubles, impaired nutrition, and other evils following. Such recognition of the wideness of the bounds of this subject of "private cleanliness" is as a matter of fact of the greatest importance with regard to the prevention of diarrhoea; for it must be understood that all the various agencies, particularly through school channels, which are at present helping to raise the general standard of cleanliness throughout the community, are at the same time tending to bring the prevention of diarrhoea rapidly within the bounds of practicability. Without these indirect aids, the

problem might have seemed quite hopeless. When we find however that with the comparatively recent institution of such agencies certain conditions of personal filth are already threatening to become rare, there is every reason to expect the best results from the education of the public in these and all kindred matters. Other points to be noted here are that the poor and dirty districts of a town have always constituted themselves, to a large extent, shelters or strongholds from which the various infectious diseases issue forth to ravage the community at large. Again, that with ideal housing reforms, the dirty habits of the people may still persist; and that while the prevalence of certain diseases may depend primarily upon defective housing conditions, others, such as diarrhoea, may have their basis rather upon the habits of the householders themselves. The two districts were particularly interesting from the fact that the housing conditions were thoroughly good, and that the dirtiness one saw there was of the essential and inexcusable kind, altogether unqualified or unexcused by poverty or other depressing causes.

It is of course useful to be able to state the results of the enquiry in some such definite manner as the above, but it is only too evident on a perusal of the preceding sections that there are too many points connected with the causation of the disease yet in doubt to allow of anything final in the way of conclusions.

A demonstration as to fly carriage would of course suggest measures against the multiplication of flies, by directing attention to the disposal of the refuse in which they breed. The points at which precautions against fly infection might be inserted, in the foregoing considerations as to preventive measures, are obvious enough to need no further remark here, except to note the necessity of securing that flies are not allowed to settle upon the lips of infants, or upon food or infectious discharges. It is evident, however, that whether the fly theory be proved or not, the importance of the above summing up as well as of other parts of the paper must still to a large extent hold good; *for flies can only become dangerous to any extent where there is laxity of the kind described in (a) and (b)*. In any case eradication procedures of the two kinds, as in malaria, should certainly go hand in hand.

Nevertheless, while the gradual inculcation and enforcement of cleanliness, and other measures, may lead to the levelling down of the greater part of the seasonal curve, yet the method of spread appears to be sufficiently independent of gross pollution to make it more than likely that a moderate incidence would still persist. Whether this does,

or does not, depend upon the peculiar ability of a carrier, such as the fly, to deal with very minute traces of infective matter is not yet certain. It is possible that, with its constant journeyings to and fro and penetration into every part of the household, the fly might cause nearly as much disease by carriage from the almost microscopical traces of infection on the basin of an apparently clean w.c., situated within a clean dwelling, as from a pan closet also so situated. Wholesale reductions in the prevalence of flies might thus be essential to the levelling down of the last part of the epidemic wave: and *the levelling down of the epidemic wave to a straight line*, as occurs in cold seasons (cf. Chart B, p. 720, Newcastle 1907), *is of course the ideal of preventive measures to be kept in mind!* On the other hand, direct personal infection may prove to be responsible for a considerable liability to spread in an ordinarily cleanly household. The concluding paragraph of Sect. VII, 2 (b), should however be here recalled: to the effect that perhaps the bulk of infection, classed under this heading, would yield to scrupulous care in washing, and in the handling and disposal of infectious discharges.

It is not of course intended to convey, in these remarks upon household dirtiness, more than that the latter is but one of several important causative agencies of diarrhoea prevalence. There is a popular sentiment that every sanitary sin brings its accompanying punishment. In the case of bad water supply and drainage the correctional disease is cholera or typhoid fever; both speedily diminishing with attention to such matters. In the case of household dirtiness, and possibly also of carelessness with fly-breeding refuse, it is perhaps diarrhoea.

In concluding this paper, I must acknowledge my indebtedness to Dr Charles Wills for permission to publish the data, and for kindly interest taken in the inception of the enquiry: also to Mr Philip J. Shacklock for his valuable meteorological data. Notwithstanding the time already taken in the preparation of the paper, the statistical analyses have proved so exacting, and the scope covered by the enquiry is so great, that I can only regard it as a hasty and premature survey of the main facts; and must claim the privilege of considerably modifying, if need be, the conclusions arrived at at a later date: it is also obvious in many places that the data will admit of much further mathematical treatment. It might be noted that the conclusions from the 1908 data were matured and considerably increased in value by the opportunities for confirmatory observations in 1909. The paper might have been fortified, had there been time, by the inclusion of many actual instances,

illustrating various important points, extracted from the original notes: these also, along with additional notes of other kinds, may possibly form the subject of a later paper. Further, in order to keep its length within reasonable bounds, anything like an attempt to review current opinions, or to provide complete references to the conclusions obtained by other writers, has been rigidly avoided throughout. References are only made to those conclusions which mark certain definite evolutionary stages, on some of which it might yet be necessary to retreat.

The collection of the data was completed two years ago, and some of the conclusions, as to personal infection and other matters, were embodied in another paper (1908). Since November 1909, when a draft of the paper was first placed in the Editor's hands, no material alterations have been made. In the meantime, a number of very valuable papers upon the same subject have appeared. Some of these have anticipated, and also unwittingly traversed arguments, and in some cases illustrations, already included in this paper. As they are not included in the bibliography, a list is given at the foot. They illustrate the trend of current opinion, and the first presents valuable morbidity data marking one of the recent notable departures in the literature, as also in our conceptions, of the disease, hitherto somewhat distorted by facts derived exclusively from the mortality data.

DAVIES. "Summer Diarrhoea and Notifications." A special report to Woolwich Borough Council. Jan., 1909.

VINCENT. "The Etiology of Zymotic Enteritis." A pamphlet. Feb., 1910.

SANDILANDS. "The Communication of Diarrhoea, etc." *Proc. Roy. Soc. Med.* Feb., 1910.

NAISH. "Summer Diarrhoea." *Public Health.* Feb., 1910.

HAMER. "Flies and Vermin." Report by the Medical Officer, London County Council. March, 1910.

NIVEN. "Summer Diarrhoea and Enteric Fever." *Proc. Roy. Soc. Med.* April, 1910.

IX. GENERAL SUMMARY OF CONCLUSIONS.

Only a general outline of the chief conclusions is given here: all important sections in the text have a summary attached.

(1) *Age Incidence: Prevalence and Fatality, etc.*

(a) *The age incidence* of the mortality and morbidity differ markedly; and it is interesting to note that so many differences exist in the disease, as studied from the latter two standpoints, that separate recognition like that accorded to two different affections is almost justified.

(b) *Prevalence and fatality*: in some midland towns the disease is a veritable scourge: 10 % of the population may be attacked during the season: the cases may number a hundred times the total of deaths.

(2) *Clinical Features, Immunity, etc.*

The clinical picture of the disease was not always complete, but association with other cases generally served to confirm the diagnosis.

The incubation period was often found to be from 6 to 30 hours in length; possibly it is sometimes longer.

The duration of attack and tendency to recurrence varied directly with the age, *i.e.* with the susceptibility of the patient. There is probably a moderate amount of acquired immunity.

The mortality, which is almost confined to infants, is determined to a striking extent by previous ill health of the patient.

(3) *Social Relations.*

Occupation was not found to produce any effect upon the incidence of the disease, except by the possible influence of the close association of the men at their work.

School attendance probably has little effect on the spread of infection, owing to the small susceptibility of those of school age.

The sharing of yards-in-common apparently had a marked influence upon the spread, or limitation, of infection.

(4) *Sanitation.*

Dirtiness of the household increases the incidence of the disease, probably through carelessness in dealing with the excreta, particularly of young children (cf. Conclusions, pp. 653—657).

The provision of water-closets, good drains, refuse receptacles, and yard paving, is in all cases of no avail where dirty and careless habits exist.

The question of w.c. versus pan closet is, moreover, probably only of minor importance; faecal infection having most to do with pollution of the interior of the household by young children not old enough to use the w.c., or by involuntary passage of stools in children of more mature years.

(5) *Food.*

(a) *Human and Cow's milk in Infant feeding.* The comparatively low incidence upon the first year of life appeared to be due to breast feeding. With the substitution of cow's milk in the second year the

maximum incidence is attained. Boiling the cow's milk gave no protection whatever.

(b) *The milk-supply* apparently plays no part, unless one of a general kind, in introducing diarrhoea into the home (cf. Conclusions, p. 682).

(c) *Infection within the home* is probably the commonest method of contracting the disease. Much infection is frequently contracted where milk is altogether excluded from the dietary (cf. Conclusions, p. 682).

(d) *Solid foods*: it is possible that, all other things being equal, no one kind of food is more likely to be a vehicle of infection than another.

(6) *Epidemiological Features.*

(a) *Personal Infection, Direct Personal Infection, Fly Carriage, Ground Infection, etc.* It appeared not improbable that the phenomena of diarrhoea prevalence are almost wholly concerned with the local evolution of various infective foci, and a piled-up mass of evidence has been presented as to the bulk of infection being derived by transmission from person to person. Further, as regards the above four sources or methods of infection a great deal of evidence was obtained for, and none against, the first three; and practically none was found exclusively supporting the last (cf. Conclusions, p. 715 *et seq.*).

(b) *Factors governing Epidemic Rise and Decline.* In relation to both the temperature and fly curves, the diarrhoea curve shows: a delayed rise: variations corresponding with their variations: and a definite falling away from their declining curves. The explosive form of the curve suggests multiplication by case-to-case infection, with gradual exhaustion of epidemic potential. The correspondences of the diarrhoea and fly curves are such as to be quite compatible with the theory of fly infection. Direct evidence, derived from the performance of certain crucial tests, is however necessary before practical adoption of the latter theory is warranted (cf. Conclusions, p. 743 *et seq.*).

(7) *Prevention and Treatment.*

Treatment. Much might be expected from a remedy such as an effective antitoxin or antibacterial serum.

Prevention. There is good reason for believing that a great deal can be accomplished by the following preventive measures:

(a) *Notification of diarrhoea sickness*: notification, of a partial kind, is shown to be practicable, and useful.

(b) *Isolation of attacked persons*: generally practicable to some slight extent.

(c) *Cleanliness in the household*: particularly with regard to avoidance, or cleanly removal, of faecal diarrhoeal pollution.

(d) *Education of the Public* as to the specific nature, and infectiousness, of the disease; as to infection through stools, etc.

(e) *Breast feeding*, wherever possible; *and proper care of food*: failing the breast a wet nurse should be procured, or a cow or goat obtained; or again in default, only milk newly drawn, and given unboiled. No reliance to be placed on boiling stale milk, *i.e.* milk which has lost its first freshness: *such milk is better not given at all.*

(f) *General sanitary measures* should be attended to: but however complete their provision, diarrhoea may rage with undiminished violence where their beneficial influence is neutralised by:—

(i) Dirty and careless habits of living; including carelessness with food.

(ii) Want of care in isolation of attacked persons, and in the handling and exposure of their stools.

It is not sufficient to merely establish good water-closets, drains, etc.: in dirty districts supervision as to their cleanly working is absolutely necessary.

A demonstration of fly carriage would call for destruction of fly-breeding grounds, and for precautions against exposure of infective discharges, as well as of food.

TABLE XXVII b. Giving some original data for the individual houses of the two districts.

In the columns H=index numbers of Houses: the numbers of Attacked Houses being in italics.
" " Dt=index figures of Dirtiness. Division into the 33 "Clean" and "Dirty" Sections of Table XX a are indicated in this column by small marks.
" " F=the Number of Children per house: italics are used in Houses containing Infants.

The Triangular Area:

H	Dt	F	H	Dt	F	H	Dt	F	H	Dt	F	H	Dt	F	H	Dt	F
1	4	10	27	2	0	53	1	3	81	2	8	107	3	3	133	2	3
2	5	4	28	2	0	54	2	1	82	3	3	108	3	2	134	2	6
3	3	2	29	2	4	55	2	5	83	-	5	109	3	2	135	2?	4
4	4	2	30	3	6	56	4	5	84	4	3	110	3	8	136	3	5
5	5	7	31	3	0	57	-	7	85	3	2	111	3	3	137	5	7
6	4	1	32	3	4	58	4	9	86	3	0	112	3	3	138	5	1
7	4	5	33	5	5	59	3	2	87	3	4	113	3	4	139	5	4
8	5	4	34	4	3	62	3	1	88	2	2	114	2	2	140	1	1
9	4	4	35	3	1	63	3	1	89	2	4	115	5	8	141	1	1
10	2	6	36	3	6	64	3	10	90	5	5	116	3	6	142	3	6
11	3	2	37	2	1	65	4	6	91	3½	1	117	3	4	143	3	7
12	3	1	38	2	5	66	-	7	92	3½	8	118	2	3	144	3	2
13	2	2	39	2	7	67	3	7	93	3	9	119	2	3	145	3	3
14	3	3	40	2	3	68	3	2	94	3½	3	120	2	2	146	2	2
15	2	4	41	3	4	69	3	6	95	3½	3	121	2	3	147	2	2
16	3	1	42	3	3	70	3	1	96	3½?	4	122	1	2	148	2	4
17	1	1	43	3	4	71	5	6	97	3½	6	123	1	2	149	1½	3
18	2	4	44	2	5	72	5	6	98	5	6	124	-	0	150	1½	1
19	1	1	45	3	3	73	4	7	99	3½	4	125	4	2	151	1½	1
20	4	9	46	1	3	74	3	4	100	3½	1	126	4	3	152	1½	1
21	4	2	47	2	4	75	3	1	101	5	2	127	4½	8	153	3?	4
22	2	5	48	3	2	76	-	4	102	4	4	128	1½	1	154	3?	6
23	4	3	49	3	4	77	5	3	103	3½?	1	129	1	1	155	1	0
24	1	3	50	3	3	78	5	2	104	3½?	1	130	1½?	1	156	1	0
25	2	4	51	3	4	79	4	4	105	4	1	131	3	5	157	1½?	0
26	2	8	52	3	3	80	3	1	106	4	9	132	2?	2	158	1	0

The Quadrilateral Area:

4	4	4	2	34	2	1	64	4	2	94	3	1	124	2	1	154	1	6	185	3	1
5	4	4	4	35	1	0	65	3	4	95	4	8	125	-	2	155	3	3	186	2	2
6	3	5	5	36	2	1	66	2	1	96	4	7	126	-	1	156	2 $\frac{1}{2}$?	2	187	2	0
7	3	2	2	37	2	2	67	3	4	97	5	6	127	2	1	157	1	6	188	-	0
8	2	1	1	38	2	3	68	2	1	98	3	7	128	2	0	158	3	3	189	3	0
9	2	3	3	39	2	2	69	-	0	99	1	2	129	2	1	159	2	1	190	-	2
10	2	0	0	40	2	2	70	5	1	100	1	3	130	-	2	160	2	4	191	-	3
11	4	2	2	41	4	2	71	4	1	101	4	2	131	3	6	161	3	1	192	-	3
12	3	2	2	42	1	5	72	3	1	102	2	7	132	2	1	162	4	6	193	1	1
13	2	2	2	43	2	1	73	2	3	103	-	3	133	2	3	163	2	1	194	1	4
14	-	4	4	44	2	0	74	3	4	104	1	3	134	2	3	164	-	2	195	1	2
15	3	1	1	45	2	0	75	-	1	105	2	2	135	2	0	165	-	0	196	2	2
16	4	5	5	46	1	3	76	-	2	106	2	2	136	2	1	166	2 $\frac{1}{2}$	2	197	1	1
17	4	5	5	47	1	2	77	2	5	107	2	2	137	2	1	167	-	1	198	1	0
18	1	3	3	48	2	1	78	-	1	108	4	5	138	5	4	168	2	1	199	1	4
19	5	0	0	49	3	5	79	3	6	109	3	4	139	3	1	169	2	3	200	1	2
20	-	4	4	50	3	1	80	2	4	110	3	2	140	2	0	170	2	2	201	1	1
21	3	4	4	51	4	3	81	2	7	111	2	1	141	2	3	171	1	3	202	1	4
22	3	5	5	52	3	1	82	3	3	112	2	1	142	2	1	172	1	4	203	-	0
23	3	1	1	53	1	1	83	2	1	113	2	0	143	2	2	173	1	0	204	1	0
24	3	3	3	54	2	2	84	3	5	114	1	5	144	3	2	174	1	4	205	1	4
25	1	2	2	55	2	4	85	2	2	115	-	0	145	2	1	175	3	5	206	1	0
26	4	2	2	56	3	1	86	2	2	116	2	1	146	2	5	176	4	1	207	-	3
27	4	2	2	57	-	3	87	2	2	117	-	3	147	2 $\frac{1}{2}$	6	177	2	3	208	1	5
28	4	4	4	58	3	6	88	2	2	118	-	2	148	2	0	178	2	2	209	1	8
29	1	2	2	59	2	2	89	4	3	119	2	3	149	2	2	179	1	4	210	1	6
30	1	2	2	60	3	4	90	3	7	120	2	0	150	-	1	180	-	0	211	3	7
31	2	3	3	61	3	8	91	1	1	121	2?	1	151	2?	2	181	1	1	212	4	4
32	3	2	2	62	2	0	92	3?	6	122	2	2	152	-	1	183	3	5	213	5	3
33	1	0	0	63	2?	2	93	2	1	123	2	4	153	-	0	184	3	9			

With regard to the index figures for *Diriness*, the above list was only used for the construction of Tables XXVII and XXVIII in the text: the other Tables are founded upon the data collected towards the middle of the season, which excludes the doubtful ones to which a query is attached above, and also a few others.

Only a few houses contained 2 children under 2 years of age, viz.: In the Triangle, Nos. 1, 26, 84 and 110: In the Quadrilateral, No. 186.

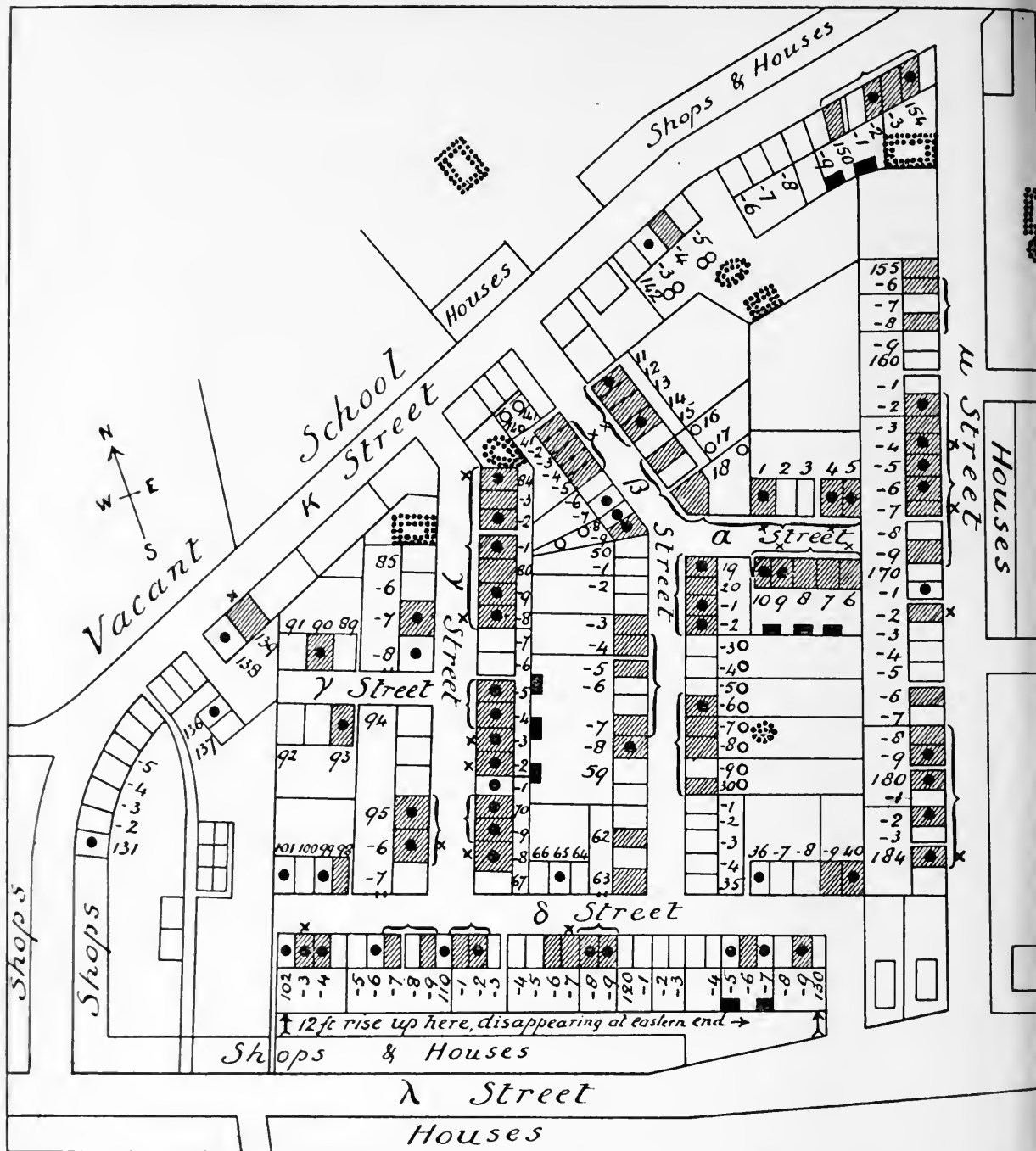













CHART I. *The "Triangular Area."* It has been necessary to simplify the Charts as much as possible. Back entrances are only indicated in a few of the largest yards; in the others the entrance was generally tunnelled through the terrace from the front street. The projections of the rear premises (cf. Chart III), which were found practically throughout both districts, are levelled off here. Shops were not visited: houses not visited are not numbered: apart from this a few other irregularities have been admitted into the numbering. Areas I, II, and III are drawn to approximately the same scale.

Explanation of signs used.

- | | | | | | |
|---|---|--------------------------------------|---|---|--------------------------------------|
|  | = | attacked houses. |  | = | privy and ashpit. |
|  | = | unattacked houses. |  | = | manure-heap. |
|  | = | houses containing infants (under 2). |  | = | stable. |
|  | = | when unnumbered = uncanvassed |  | = | brackets enclosing "time and place |
|  | = | houses, or data are not complete. | | = | groups" of Chart VII. |
|  | = | pan (pail) closets. |  | = | crosses are placed in the streets in |
| | | | | | front of houses in which cases had |
| | | | | | already occurred before July 3. |

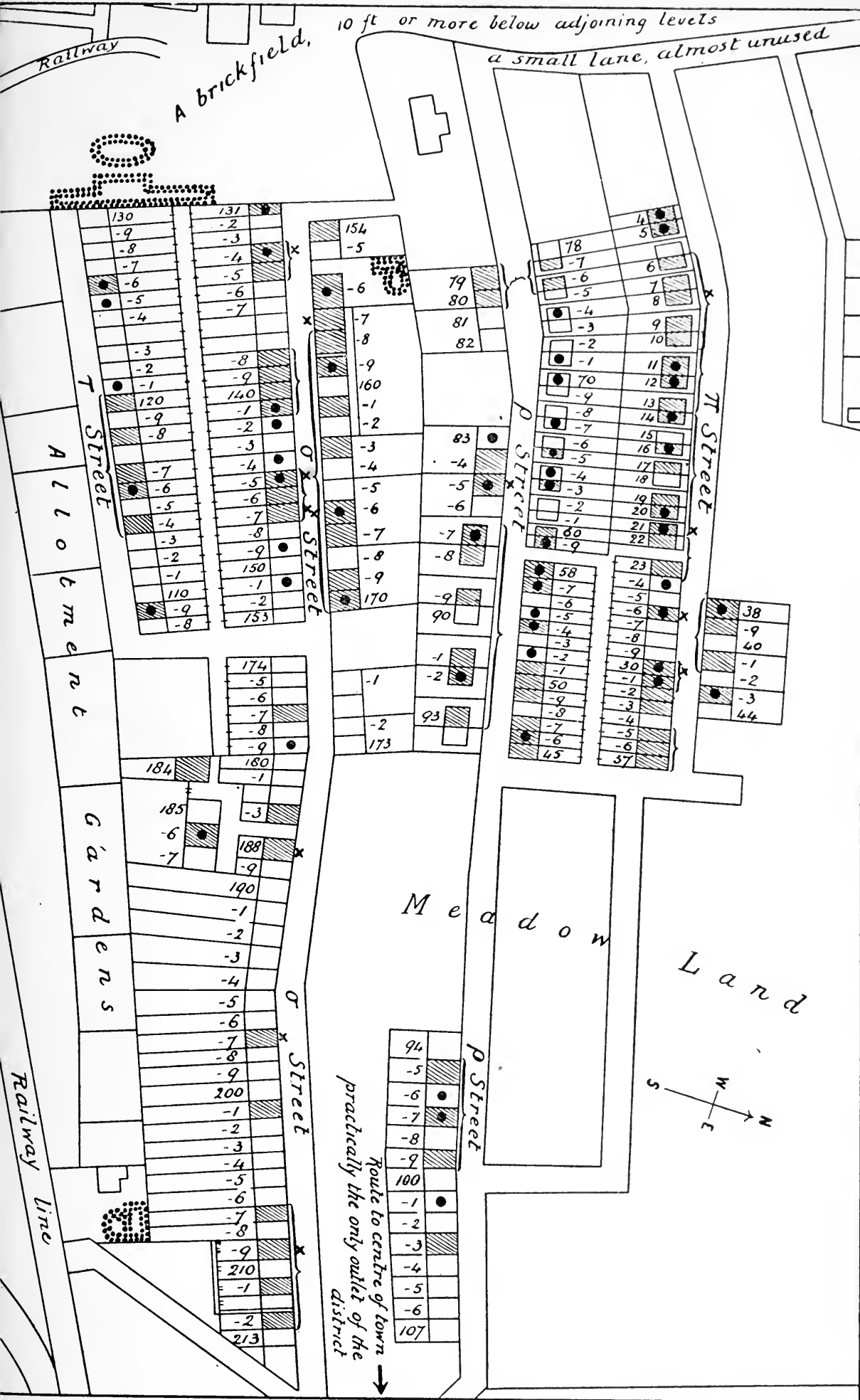


CHART II. The "Quadrilateral Area." (For explanatory notes see Chart I.) Open country bounds the district on the west and north, and a wide railway reserve on the south.

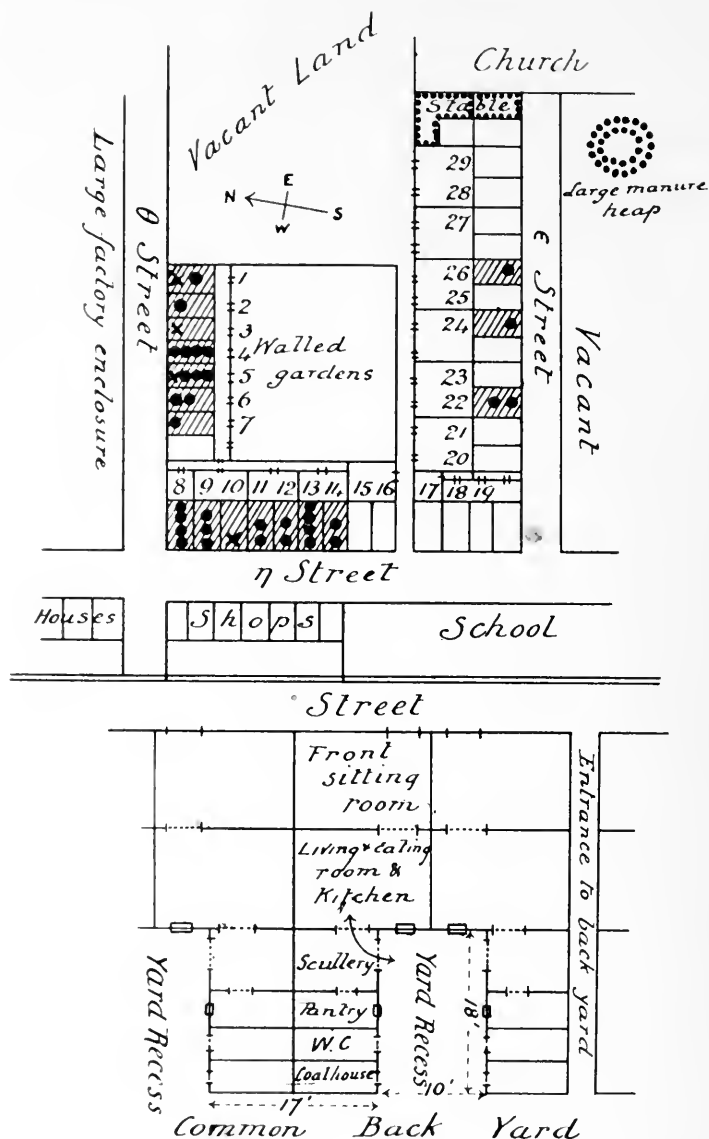


CHART III. (a) "Area III." Large fly-swarms were observed entering and spreading through the district from a large heap of horse-manure at the S.E. corner. Diarrhoea however appeared first in, and was almost confined to, the N.W. corner. No stables or breeding grounds for flies could be found anywhere around this corner within, at least, twice the distance separating it from the manure-heap at the S.E. corner. Other dwelling houses were also absent on all sides for considerable distances except immediately opposite the N.W. corner, from which direction then diarrhoea infection, if derived from outside, must have entered the district; *i.e.* against the direction of the fly movements. Infection then, if carried between houses by flies, was not at least brought with them from their breeding ground. They were all new brick houses, with w.c's. Greater cleanliness and fewness of children did not explain the relative immunity of the houses in the S.E. corner. Cf. also Chart IV. The signs used are the same as in Chart I, except that • = a diarrhoea case, × = a diarrhoea case occurring before July 18.

(b) Plan of houses in a typical terrace. Note the narrow recess at the rear, into which the kitchen, scullery, pantry, and w.c. of each pair of adjoining houses open. The measurements are merely typical.

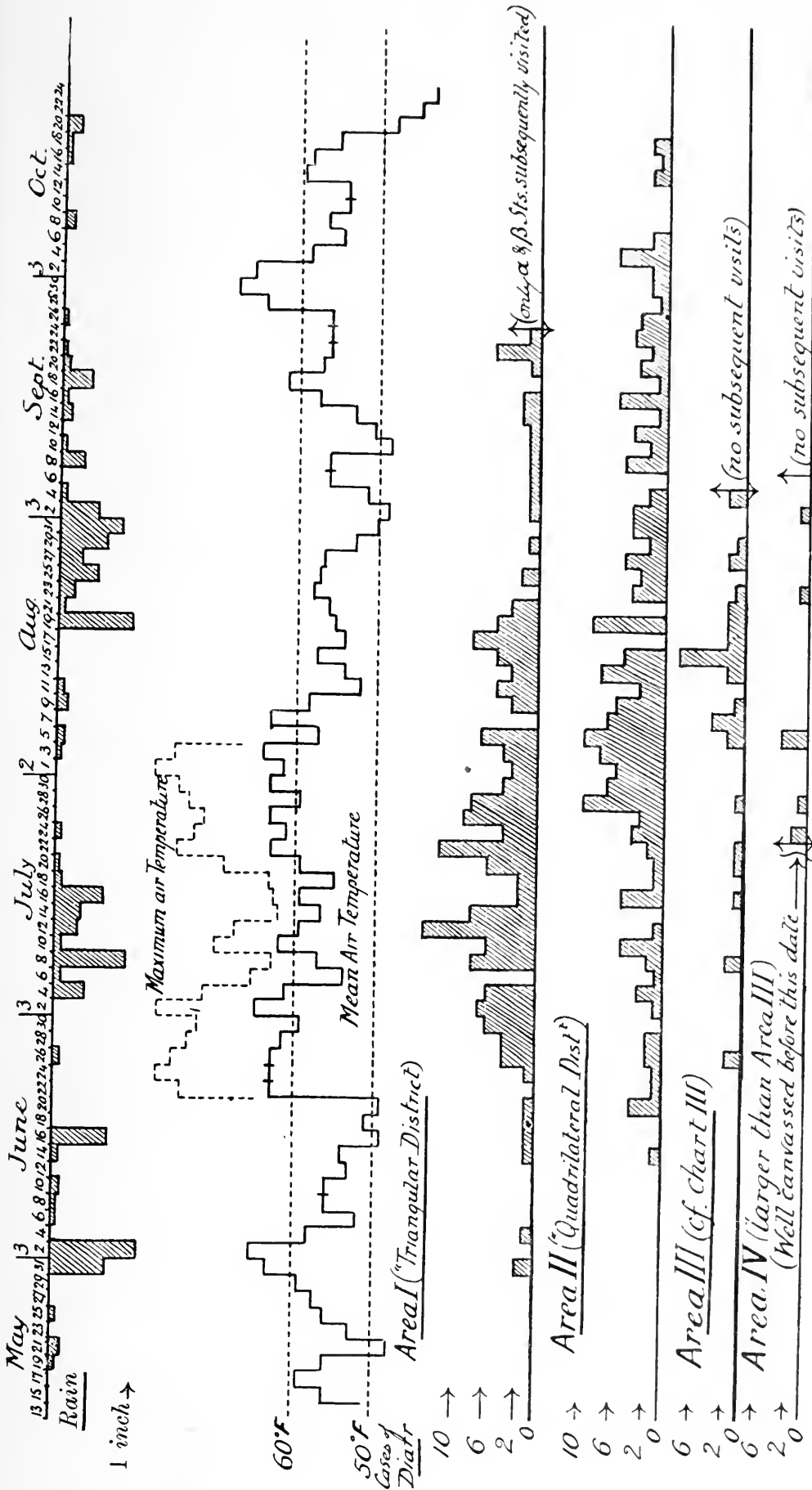


CHART IV. 2-day records of Onsets of Diarrhoea Attacks, and of Rainfall; 2-day averages of Mean and Maximum Air Temperatures: Mansfield, 1908.

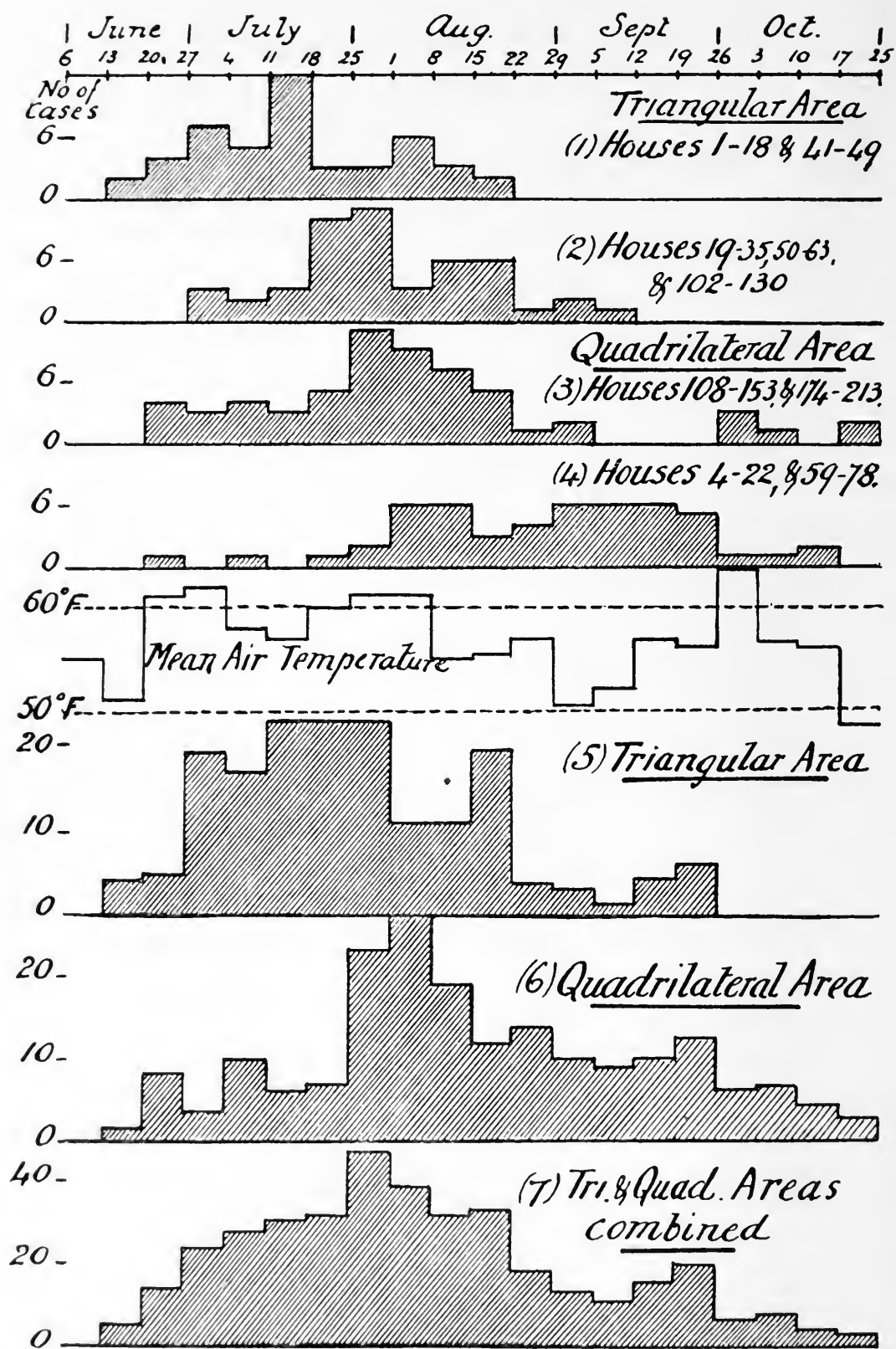


CHART V. Weekly records of Onsets of Diarrhoea Attacks, and of the Mean Air Temperature: 1908. Irregular evolution of the epidemic in different districts, epidemic exhaustion, and variations with temperature are well illustrated.

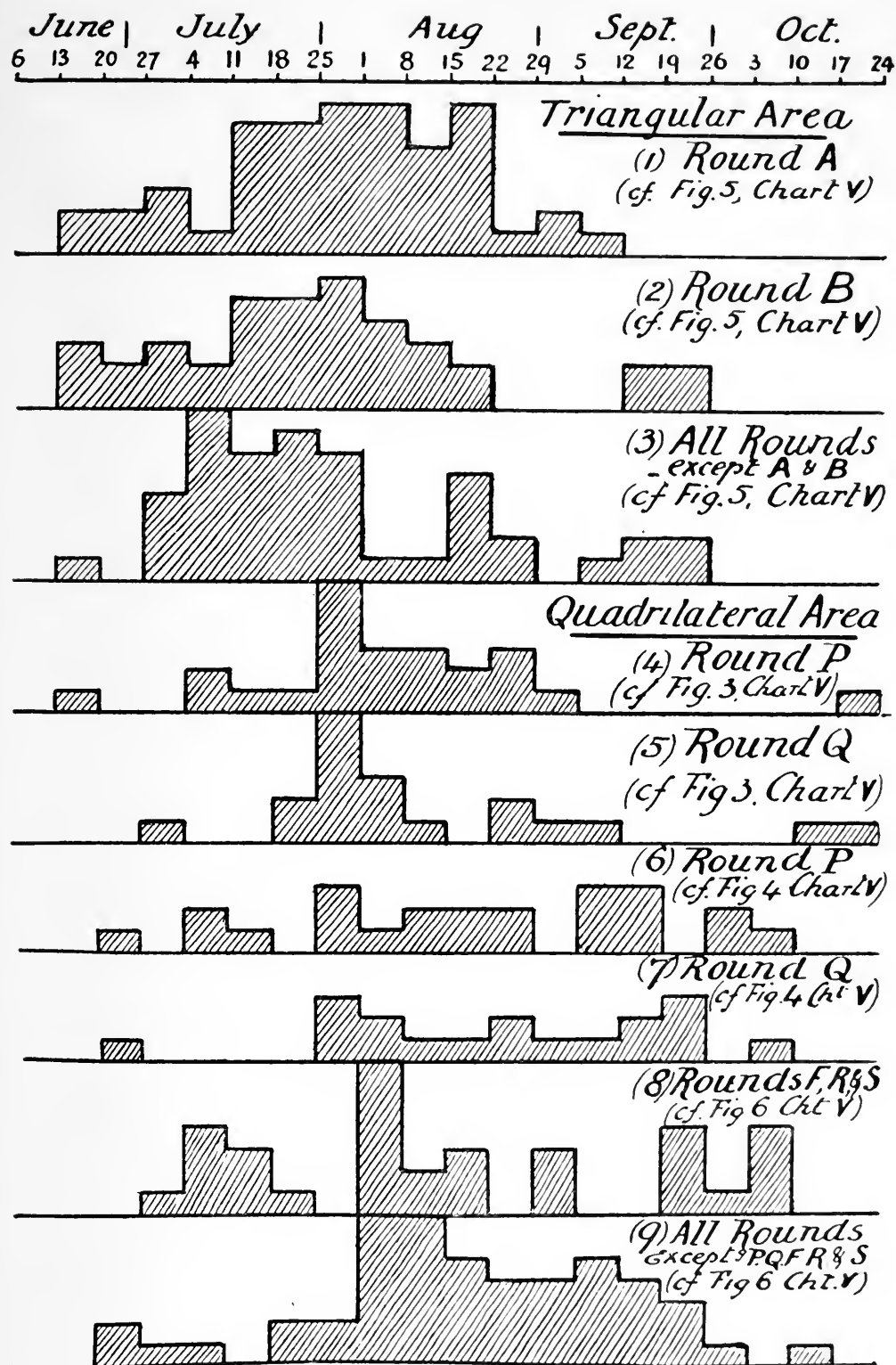


CHART VI. The number of attacks per week in various Milk-Rounds. Comparing the contour of the above figures with that of the corresponding figures in Chart V, and after making due allowances, remarkable correspondences in form are visible, the curve of attacks in any milk-round thus conforming with complete passivity to the curve of attacks in the whole section or district in which the milk-round is located; no part being therefore played by the Milk-Supply, unless one of a general kind, in the distribution of diarrhoea attacks. No suspicious clumping of cases was visible even when the daily records in each of the 21 rounds were separately examined. Rounds P and Q, to which special interest attaches, have been specially analysed. Figs. 6 and 7 include houses 4—78, and Figs. 4 and 5 the rest of the district. The drawing is to the same scale throughout: the maximum weeks of Fig. 1 are of 7 cases each. Cf. also Table XXXI.

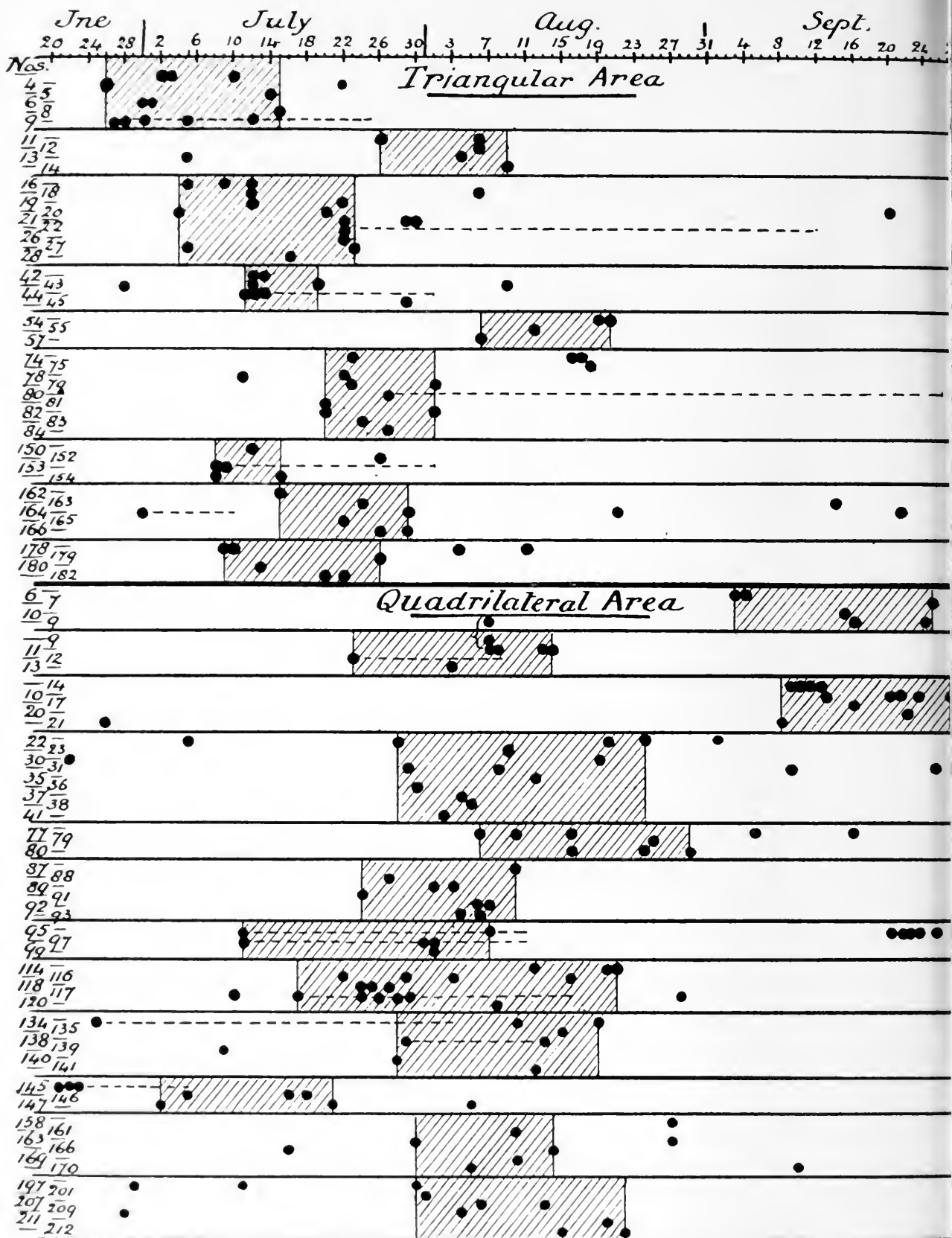


CHART VII. Showing Grouping of diarrhoea cases in point of Time, as well as of Place. The dots give the dates of onset of attacks when referred to the calendar at the top; the index numbers of the houses in which the attacks occur are also given in the left-hand margin: in a few cases, where of special interest, the length of the attacks is indicated by dotted lines. Cf. Charts I and II.

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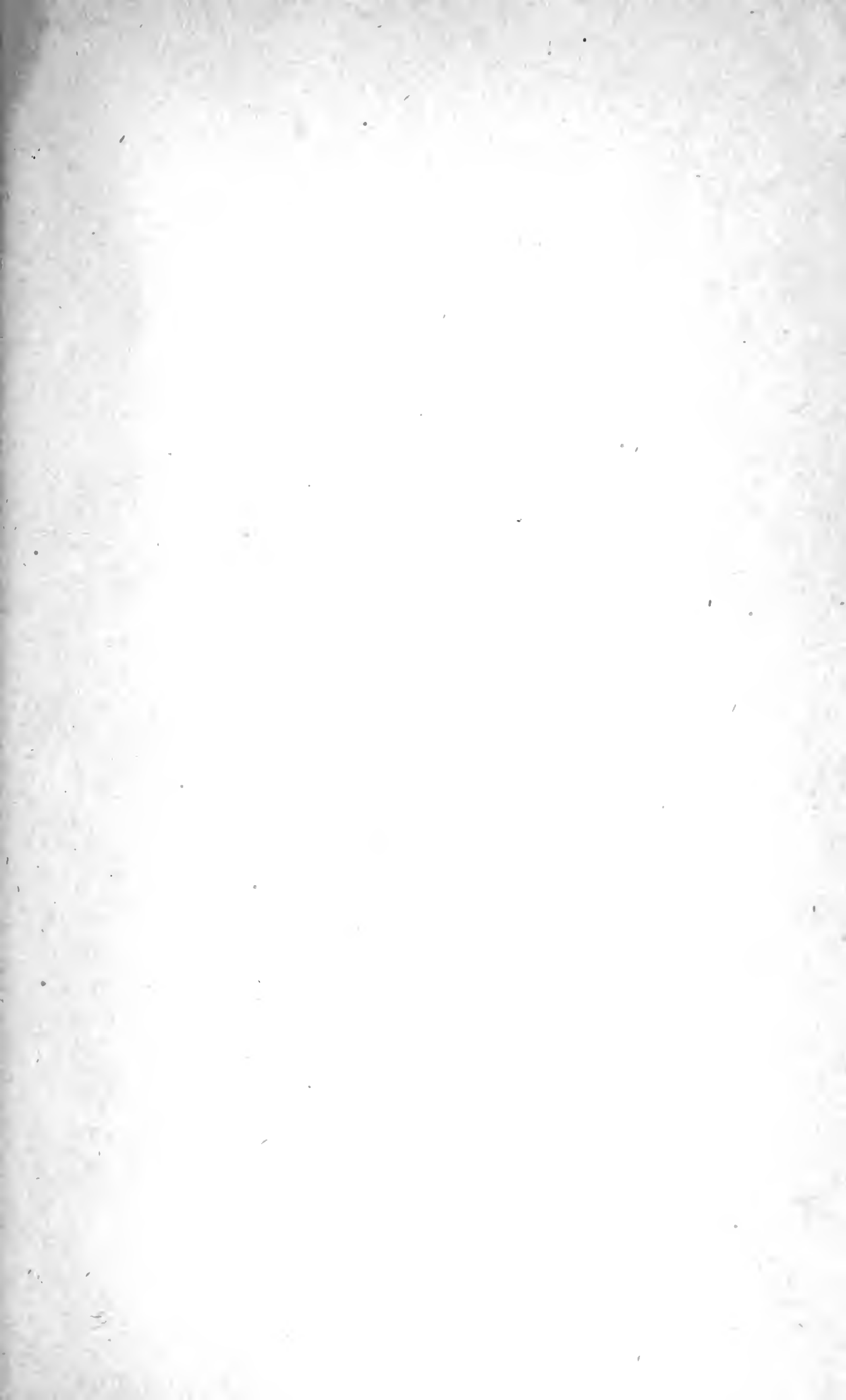
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